

Behavioral Variations Caused by Methyl Mercury Exposure -the Construction of a Diagnostic Instrument

by Jan Hellberg



LUND UNIVERSITY SWEDEN
DEPARTMENT OF PSYCHOLOGY
DISSERTATION SERIES

1978



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41553421_0027

Behavioral Variations Caused by Methyl Mercury Exposure
- the Construction of a Diagnostic Instrument.

av

Jan Hellberg
Fil.kand., Ssk

A k a d e m i s k a v h a n d l i n g

som för avläggande av filosofie doktors-
examen vid samhällsvetenskapliga fakulte-
ten vid universitetet i Lund kommer att
offentligen försvaras i Carolinasalen,
Kungshuset, Lundagård, onsdagen den 12
april 1978 kl 16.15.

Dokumentutgivare

04T0 Psykologiska inst., Lunds univ.

Handläggare

06T0

Författare

08T0 Jan Hellberg

Dokumentnamn

04T4 Rapport

Utgivningsdatum

06T4 Apr 1978

Dokumentbeteckning

04T6 LUSADG/(SAPS-
1020)/1-14/(1978)

Ärendebeteckning

06T6 Doktorsavhandl.

10T4

Statens medicinska forskningsråd.
Medicinska fakulteten vid Lunds
universitet.

Dokumenttitel och undertitel

18T0

Behavioral Variations Caused by Methyl Mercury Exposure
- the Construction of a Diagnostic Instrument.

Referat (sammandrag)

26T0

Inside a major project concerning the toxicity of Methyl Mercury (MeHg) attempts have been made to construct a diagnostic instrument for the detection of pre-clinical signs of MeHg poisoning following oral exposure to mercury. During exposure the investigations were performed on squirrel monkeys (Saimiri sciureus). It was shown that learning-set, motivation and foveal discrimination was not affected prior to any clinical signs. An apparatus for determination of critical flicker fusion intensity (cffl) in scotopic vision in the monkeys was then constructed and by testing the monkeys during exposure it was shown that cffi increased as blood level of MeHg reached toxic levels and that it happened prior to any clinical signs. During the tests flicker-rate was held constant at 10 cps. The lesions in the monkey brain were localized cortically and most pronounced in and around the visual centers. Based on the principles established, an apparatus for determination of cffi for humans was constructed. Tests were performed in order to establish normative values for the un-exposed population and a standardized test procedure.

Referat skrivet av

42T0

förf.

Förslag till ytterligare nyckelord

44T0 Behavioral variations. Cortical lesions. Sensory threshold. Cffi vs. different light wavelengths. Cffi vs. fatigue.

Klassifikationssystem och -klass(er)

50T0 Physiological intervention. General psychology.

Indextermer (ange källa)

58T0 Lesions. Research methods & Apparatus & Computer applications (Psychological abstracts).

Omfång

56T0 71 pages

Språk

58T0 english

Övriga bibliografiska uppgifter

56T2 Incorporated in the dissertation series of the Department of Psychology. No 1020. Edition: 220 copies.

Sekretessuppgifter

60T0

ISSN

60T4

ISBN

60T6

Dokumentet kan erhållas från

62T0 Psykologiska institutionen
Lunds universitet
Paradisgatan 5, 223 50 LUND

Mottagarens uppgifter

62T4

Pris

66T0

RAPPORT CODEN: LUSADG/(SAPS-1020)/1-14/(1978)

Behavioral Variations Caused by Methyl Mercury Exposure -the Construction of a Diagnostic Instrument

by Jan Hellberg



DISSERTATION SUMMARY
APRIL 1978
DEPARTMENT OF PSYCHOLOGY
LUND UNIVERSITY
PARADISGATAN 5
S-223 50 LUND, SWEDEN



This summary is based on five reports, referred to in the following as I, II, III, IV and V. The reports are:

- I. Hellberg, J., Nyström, M. and Nilsson, O. (1971). Metod att testa visuell och sensorisk diskrimination, retention, motorisk koordination och inlärning hos Dödskallean (Saimiri sciureus). (A method to test the visual and tactile discrimination, retention, motor coordination and learning abilities of the Squirrel monkey (Saimiri sciureus)). Nordisk Hygienisk Tidskrift, Band LII, 14-21.
- II. Hellberg, J. and Nyström, M. (1972). The Influence of Methyl Mercury Exposure on Learning-set Behavior of Squirrel Monkeys (Saimiri sciureus). Psychol. Res. Bull., Lund University, XII, no 8.
- III. Hellberg, J. and Hellström, J. (1977). Changes in Scotopic Flicker Fusion Intensity (cffi) in Squirrel Monkeys Exposed to Methyl Mercury. Psychol. Res. Bull., Lund University, XVII, no 12.
- IV. Hellberg, J. (1978). A Method for Measurement of Critical Flicker Fusion Intensity in Scotopic Vision. Psychol. Res. Bull., Lund University, XVIII, no 1.
- V. Hellberg, J. (1978). The Influence of Fatigue and Different Wavelengths of Light on Critical Flicker Fusion Intensity in Scotopic Vision. Psychol. Res. Bull., Lund University, XVIII, no 2.

B a c k g r o u n d

In connection with a major project concerning the toxicity of methyl mercury (MeHg), it was asked whether it was possible to use variations in the behavior of the individual exposed to MeHg as an indication of brain lesions before any conventional clinical signs appeared. The plan was to start with experiments on animals, and with the results obtained in those experiments as a basis, to construct a diagnostic instrument for use on humans.

The reason for stressing the necessity of tracing pre-clinical signs

is the fact that clinical symptoms following MeHg exposure are irreversible. The cause of the symptoms are cortical lesions (Berlin, Nordberg & Hellberg, 1973; Grant, 1973; Berlin et al., 1975).

In the first experiments Squirrel monkeys (*Saimiri sciureus*) were used. This monkey was considered to be especially suitable for this kind of investigation since it has a central nervous system that shows great similarities to that of man as far as reactions to MeHg exposure are concerned (Nordberg, Berlin & Grant, 1971; Berlin et al., 1973).

It was known from experiments with MeHg exposure that the mercury is distributed fairly evenly throughout the CNS of the monkey during the period immediately following oral intake. By means of autoradiography it has been established that both cortical and sub-cortical tissue as well as basal ganglia showed the same concentration. This picture underwent a radical change if the distribution was studied some time after the intake of MeHg. It was then found that the remaining mercury was concentrated to cortical tissue and was most abundant in and around the visual cortex (Berlin, Nordberg & Hellberg, 1973; Grant, 1973; Berlin et al., 1975).

Histological and pathological examinations of brain tissue showed that a pronounced cell death was at hand. Primarily the glial cells were destroyed and, secondarily, as a consequence, and after some time, the neurons were also affected (Nordberg et al., 1971; Grant, 1973). At a stage where the glial cells alone were affected, it can be assumed that the neurons will show functional disturbances caused by lack of oxygen and nutrition (Julien, 1975). If the exposure is terminated at this stage the damage ought to be reversible, since the glial cells have regenerative capacity - something the neurons lack.

When the facts described above were combined it was possible to analyze the problem of how to trace pre-clinical behavioral changes in the monkeys exposed. The development of clinical signs parallels that in man: visual disturbances, ataxy that, in an initial stage, affects the finer motor functions, and later loss of balance. As a

gross trend a worsening of the symptoms can be seen that leads to blindness, severe functional disturbances and ultimately death (Nordberg, Berlin & Grant, 1971). As a consequence aspects of behavior containing components of both visual and motor sequences ought to form the basis on which a test situation can be formulated.

Formulating a test situation.

Injuries caused by physical force and affecting the brain locally and with cell destruction, can give rise to distinct symptoms. At the same time this opens the possibility of determining the locality and the degree of the injury. This is especially the case if the area destroyed lies in, or includes part of, a sensory or motor center. The symptoms can be distinct even if areas in the brain-stem are affected. For this reasoning to be valid it is assumed that the injury include only a small portion of the brain tissue.

Injuries caused by mercury poisoning cannot be described as small and well localized. As mentioned earlier, the stage immediately following oral exposure showed a distribution picture where all parts of the CNS contained mercury in about the same concentration. If the dose is strong enough to affect the functioning of the system, effects ought to be found on all levels. Later on, when the mercury is concentrated cortically, and especially in the visual cortex, disturbances of the visual functions should be the most pronounced signs. But in this case the symptoms could be expected to involve several functions.

Learning processes, especially those involved in the establishment of long time memory, involve subcortical activities that are of vital importance. The Hippocampal nuclei are directly involved in these processes (Hubbard, p. 100, 1975), and consequently learning may be affected if the activity of these basal ganglia is suppressed.

The neurons of the brain are protected by the so-called Blood-Brain-barrier. Functionally this means that the neurons have no direct contact with the blood vessels, and that exchange of oxygen and nutrients goes via the glial cells. Thus the latter are indirectly involved in the nervous activity. MeHg primarily destroys these

cells and as a consequence, secondarily, the neurons are also destroyed. In a stage where mercury is found diffusely distributed and in concentrations strong enough to be harmful, it can be expected to cause a general inhibitory effect noticable in prolonged reaction times (latencies), decreased level of general activity and learning capacity and also changes in motivational status.

As mentioned above ataxies, most pronounced in finer motor behavior, are early and specific symptoms of mercury poisoning. By a closer study of a well defined motor sequence, it might be possible to detect early malfunctions. This *a priori* assumption that changes will be in the direction of malfunctions, is based on the fact that MeHg is clearly toxic.

The visual system is affected by injuries in the visual cortex. During periods immediately following oral exposure to single doses, it is possible that sub-cortical areas are also affected. This could for instance include the lateral geniculate bodies, the main relay stations in the visual pathways. In spite of the fact that the MeHg-induced injuries are centrally localized and that the eye and retina function normally, it ought to be possible to use conventional discriminatory tasks as indicators of a functional decrease of the visual system.

In summary the above reasoning led to the conclusion that the monkeys/experimental animals should be made to act in a situation where visual discrimination, learning, latency and a well defined motor sequence could be studied. This is a situation most adequately described as a standardized test. By studying latency two goals were reached. First, an objective measurement expressed as "reaction time" and measured by electrical timing was obtained and secondly indirect information as to the motivational status of the animal could be derived from the results.

Report I describes the test situation that was formulated. The final design was decided upon after experiments with an exact copy of the WGTA (Wisconsin General Test Apparatus, Harlow, 1949). The monkeys however showed acute stress symptoms when they were moved

from their living cage to the WGTA. It was then decided to construct a modified version. In an attempt to reduce the level of stress, the experimental animals were allowed to live among other monkeys in a common cage housing about 20 animals. When the test was performed a special lock was fastened on the front of the big cage and attempts were made to train the experimental animals to enter the lock while the other animals were to stay in the cage. This did not work since all the monkeys fought to get into the lock and get the reward.

At the end a compromise was made. The experimental animals were each permanently placed in separate cages. When testing took place the apparatus was fastened on the front of the cage. This compromise gave a somewhat lesser degree of control. At the same time functional reasons such as the procedures connected with MeHg exposure made it necessary. At those occasions it was necessary to remove the monkeys from their cage for oral exposure, weighing and blood sampling at regular intervals about twice a week.

As can be seen from Report I the final test situation was relatively complicated. During apparatus training it was evident that a typical learning-set development took place. When it was formed the monkeys functioned with great skill in the test. The process of new learning required of the monkeys in each new test session could consequently be studied as a well defined phenomenon.

During work towards a standardization of the test procedure, it was gradually felt that the study of eventual effects which MeHg exposure could have on the learning-set might come in as an extra variable providing yet a chance to detect pre-clinical effects.

Report II contains the results from tests with monkeys exposed to mercury. The number of monkeys exposed is small. The reason for this is twofold: in the first place monkeys are relatively exclusive experimental animals both in that they are expensive and require a lot of time from the personnel that keep them. Secondly there is a lot of work connected with the exposure in the form of exposing the animal in a strictly controlled way, taking blood samples and

analyzing them. Furthermore the exposure had, in some cases, to be very prolonged in order to copy a situation that might, for example, arise in industry. In one case exposure continued for almost a year. All these factors put together made it necessary to use only a small number of animals.

The results showed that what best can be described as "general functions", i.e. latency, motivational status, learning and stability of learning-set, were not affected in a way that could be coupled to pre-clinical changes in the brains of the monkeys. At the point when the results were affected in the test, it was also possible to observe clinical symptoms. These typically consisted of a gradual, concentric constriction of the visual field and ataxies of the fingers. The latter were interpreted as loss of feed-back caused by lesions in the sensory cortex.

All test sessions were filmed from the moment analysis showed that the blood level of MeHg had reached a toxic level. This made possible a thorough study of the development of clinical symptoms and behavior in the test situation. These studies showed that the peripheral visual field was affected well in advance of any decrease in ability to discriminate. As a matter of fact this ability prevailed up to the moment immediately prior to blindness.

In spite of severe disturbances of visual functions, pronounced ataxies and tendencies to loss of balance, the monkeys continued to perform in the test. Learning-set and motivational status seemed totally unaffected. As long as foveal vision was intact and the monkeys could see the stimuli they could also discriminate between them and learn which one contained the reinforcement.

In summary these results led to the conclusion that it is not possible to make an early diagnosis by studying these general functions. The results also confirmed that the development of symptoms in monkeys parallels that in man. This is especially evident when the visual functions are considered. It was further concluded that, in order to make possible an objective and standardized pre-clinical diagnosis, the early and pronounced disturbances of sensory func-

tions, especially the visual ones, should be made the subject for further investigations.

Report III describes a method for measuring critical flicker fusion intensity (cffi) for scotopic vision (night vision) in monkeys and gives the results from measurements of cffi on monkeys exposed to MeHg.

The reason for the use of measurements of cffi for scotopic vision was primarily that the results described in Report II gave reason to concentrate on visual functions. The early, gradual constriction of the visual field following exposure to mercury, pointed to some kind of perimetry, the aim being to establish the width of the visual field and then to measure it continuously during exposure to see if any changes occurred.

In a pilot study an apparatus for conventional perimetry was constructed. It was built in the form of a semi-circular ramp with a great number of small lights and a chair in the center of the circle. The lights gave white light of an intensity above the lower threshold for photopic (daylight) vision. The monkey was placed in the chair and its head was moderately fixated and training was aimed at making the monkey give a distinct response as soon as any of the lights were turned on. The response was meant to be a conditioned response according to a classical conditioning paradigm. This method was abandoned primarily because of difficulties in making the monkey give a distinctive response.

During discussion with Dr Carroll Wodworth, University of Iowa, interest was focussed on the possibility of using a sensory threshold as an indirect measurement of changes in the visual field. At low light intensities it is the rods in the retina that are active (scotopic vision). If the intensity of a flickering light, with a flicker rate constantly held at, for instance, 10 cps, is gradually decreased a point where an observer no longer can perceive the flicker is reached. At the flicker rate 10 cps the light intensity is well below the upper threshold for scotopic vision. This intensity, when the perceived quality of the light changes, is called the

critical flicker fusion intensity or cffi. When flicker rate changes a corresponding change of cffi occurs. As cffi at 10 cps lies within scotopic vision it is of special interest in this case since the periferal parts of the retina are densely packed with rods and contain very few cones. When this was considered against the fact that the periferal visual field is affected in an early stage of mercury poisoning, cffi at 10 cps became very interesting.

As pointed out earlier the injuries caused by MeHg are cortical. In spite of this it ought to be possible to use conventional stimulus situations, establish sensory thresholds, to study them, and if changes occur to draw conclusions about disturbances on the cortical level. Of course this must be coupled to knowledge of the histopathological picture of the cortical area in question. Damage caused by MeHg in Squirrel monkeys is thoroughly investigated and described (Grant, 1973).

By studying cffi for scotopic vision no information about the periferal visual field in terms of width expressed in degrees can be derived. However, since the periferal parts of the retina are the basis for periferal vision, it was felt that a sensory threshold directly connected to the rods would serve as a reliable indicator of any kind of malfunctions affecting the periferal visual field. This was especially the case since at no time have absolute values been stressed. Emphasis has instead been placed on the concept of change.

The apparatus described in Report III was constructed according to this reasoning. By testing monkeys before and during exposure to MeHg with the apparatus it was shown that it was possible to determine cffi for scotopic vision and to study it over a long period of time. The cffi for the monkeys was very stable over time and it was shown that when changes occurred they were correlated to toxic blood levels of mercury. Furthermore it was shown that these changes occurred well before any clinical signs could be observed. Another and important finding was that the earliest changes - towards an increase in cffi - were of a reversible nature. Compared to what was said in the introductory discussion, the results pointed to a

possible registration of effects emanating from the glial cells. If exposure is terminated at this stage there is a possibility that the system can return to a state of normal functioning.

These results were considered promising enough for the next step towards the construction of a diagnostic instrument for use on humans to be taken. The instrument was to be constructed according to the same principles, that is, measurements of cffi at a fixed flicker rate of 10 cps and variable light intensities within the range for scotopic vision. Further the criteria of automatization, simple handling and instructions were to be met. These criteria were justified considered against a situation where the instrument might have to be used for epidemiological investigations in the field.

Report IV describes the apparatus and the method designed to meet the above mentioned criteria. The main goal for the investigation was to standardize the test procedure and to obtain normative measurements of cffi from the normal, non-exposed population.

As can be seen from the report the apparatus worked very well. Further the results gave reason to suggest a standardized test procedure that is simpler than the one used in the investigation. It was possible to exclude certain time consuming stages without any loss of information. The variations, both intra- and interindividual, that were found, were of a magnitude that did not interfere with the predicted changes caused by mercury poisoning (Berlin et al., 1976).

The sample tested was relatively large, but as a further control another sample was tested. It contained the same number of individuals and was also from a non-exposed population. Three questions sought an answer. Firstly the simplified test procedure was to be tried, secondly further information concerning what effects fatigue, within normal boundaries, might have on cffi was to be investigated and thirdly it was asked if it was possible to increase the sensitivity of the apparatus by changing the wavelength of the stimulus light towards the optimum for rod sensitivity.

The third question arises from the fact that the apparatus was originally fitted with light diodes because they gave exact control over intensity and flicker rate. At the same time that meant adding a limitation since the shortest wavelength any available diode emitted was 5650 A while the optimum for rod sensitivity corresponds to 5000 A.

Report V shows that fatigue within normal boundaries, in the investigation defined as the fatigue produced by a full working day, did not affect cffi. Further the simplified test procedure was found satisfactory.

The part of the investigation that concerned the influence of different wavelengths on cffi made necessary a minor modification of the apparatus. It meant fitting another complimentary diode emitting a longer wavelength (5900 A). Since no diodes emitting wavelengths shorter than 5650 A were available, the only method that remained was to compare cffi₅₆₅₀ with cffi₅₉₀₀ and then make an extrapolation in order to predict cffi₅₀₀₀. This extrapolation was made by relating the two sets of data from the tests to an absorption spectrum for rhodopsin, which is the light pigment of the rods, and then compare the result with the point in the spectrum corresponding to 5000 A. This showed that a considerable increase in sensitivity of the instrument would follow a change to a light source emitting 5000 A.

If the results from Reports IV and V are summarized, it is evident that an instrument that meets the criteria mentioned earlier has been constructed. The automatic components are reliable, the test procedure is simple and requires very little instruction and the results show a satisfactory stability. The inter- and intraindividual variations are of a magnitude that do not interfere with the possibilities of tracing pathological changes following MeHg exposure. If it is considered necessary to increase the sensitivity of the instrument a considerable increase will follow a change of the wavelength of the stimulus light towards 5000 A. However, the latter would require a major modification of the stimulus array while the test procedure and general principles would remain the same.

Concluding remarks

The research described in this summary and presented in the five reports can be considered typical for work towards a solution of a well defined practical problem. In this case the task was to construct a diagnostic instrument for use on human subjects exposed orally to methyl mercury.

It was evident from the beginning that no experiments with mercury exposure could be undertaken with human subjects. That left only one way open, namely the use of animals as subjects. In the wide range of laboratory animals of different species, the choice fell on a monkey: Squirrel monkey (*Saimiri sciureus*). Among the reasons for this choice was the fact that the Squirrel monkey has a relatively highly developed nervous system. Further it is widely used in experiments and consequently a lot of information concerning different aspects of its behavior and physiology is available. Another important factor was that it had been used in previous experiments on mercury exposure and that the results from these showed that the monkey developed symptoms that paralleled those described in man (Nordberg et al., 1971). The latter was considered very important because it opened possibilities for generalizations to the human situation.

At not time during the experiments with the monkeys have the absolute values of the measurements been stressed. The main interest has been focussed on stability and change. It was considered significant if measurements that had been stable over long periods of time suddenly changed, either in the direction of increase or decrease, and this change could be correlated to aspects of the mercury exposure. It was also felt that by concentrating on the phenomenon of change, it was safe to generalize to the human situation.

This reasoning led, relatively early in the work, to concentration on a certain sensory threshold, cffi, as the subject for further studies. In both man and monkey the first signs of mercury poisoning concern vision which explains the choice of sensory threshold. Furthermore the histo-pathological picture is about the same in both cases.

When it was evident that changes in cffi in scotopic vision was a significant, pre-clinical sign of mercury poisoning in the monkeys, all reasons spoke in favor of the application of the method on human subjects. In some aspects it was even considered to be of further advantage in the human situation. The main such advantage being that the measurement of a sensory threshold is as near an objective measurement as it is possible to get. This was considered important since a prolonged exposure to MeHg in low doses might give rise to a very slow and gradual destruction of cortical tissue. During such a process a successive adaptation to the slow decrease of visual functions can make it impossible for the individual to detect and verbalize the change. In such a case an objective measurement is necessary. If the method of measurement is sensitive enough, it should be possible to register changes of the sensory threshold before the individual experiences any decrease.

I believe that the diagnostic instrument that is the result of this work meets the criterion of giving the possibility of tracing pre-clinical changes in visual functions caused by methyl mercury poisoning. It is my wish that these changes can be traced in a stage when they are still of a reversible nature.

A c k n o w l e d g e m e n t s

I wish to express my gratitude to professor Maths Berlin and the entire staff of the Department of Environmental Health, Lund University, who made this work possible by inviting me as a participant in the project, and by giving constant support during the different stages.

Special thanks are due to Ingrid Hjelm, Olle Nilsson and Lisa Tengquist who spent many, many hours trying to get the monkeys to cooperate.

I also wish to express my thanks to Jonas Hellström, Mats Almer Birgitta Hagberg-Sibbmark and Arne Svenonius who by their work within the project gave valuable help by empirically testing my ideas.

Funds were made available by the Swedish Medical Research Council and the Medical Faculty of Lund University.

Lastly but not the least I wish to thank Dr Mats Nyström for his invaluable advise and support from the very start.

Lund, April 1978

Jan Hellberg

References

- Berlin, M., Nordberg, G.F. & Hellberg, J. (1973). The Uptake and Distribution of Methyl Mercury in the Brain of Saimiri Sciureus in Relation to Behavioral and Morphological Changes. In M. Miller and T. Clarkson (Eds), Mercury, Mercurials and Mercaptans. Springfield: Charles C. Thomas, Pp. 187-208.
- Berlin, M., Grant, C.A., Hellberg, J., Hellström, J. & Schütz, A. (1975). Neurotoxicity of Methyl Mercury in Squirrel Monkeys. Arch. Environ. Health, 30, 340-348.
- Berlin, M., Gage, J., Grant, C.A., Hellberg, J. & Skerfving, S. (1976). Fortsatta undersökningar över metylkvicksilvers neurotoxicitet oeg biotransformation samt utarbetande av metoder för studier av toxiska effekter. Forskningsplan och vetenskaplig rapport (Swedish Medical Research Council, project B75-14X-2081-09), Department of Environmental Health, Lund University.
- Grant, C.A. (1973). Pathology of Experimental Methyl Mercury Intoxication: Some Problems of Exposure and Response. In M. Miller and T. Clarkson (Eds), Mercury, Mercurials and Mercaptans, Springfield: Charles C. Thomas, Pp. 249-312.
- Harlow, H.F. (1949). The Formation of Learning Sets. Psychol. Review, 56, 51-65.
- Hubbard, J.I. (1975). The Biological Basis of Mental Activity. Reading, Massachusetts: Addison-Wesley.
- Julien, R.M. (1975). A Primer of Drug Action. San Francisco: W.H. Freeman and Company.
- Nordberg, G.F., Berlin, M. & Grant, C.A. (1971). Methyl Mercury in the Monkey. In Proceedings of the 16th International Congress of Occupational Health, Tokyo, Sept. 22-27, 1969, Pp. 234-237.

Särtryck av Hygienisk Tidskrift, Band LII 1971, sid. 14—21

**Metod att testa visuell och sensorisk diskrimination, retention,
motorisk koordination och inläring hos Dödskaalleapa
(Saimiri squireus) ***

*av Fil. kand. Jan Hellberg, fil. dr. Mats Nyström och
instrumentmakare Olle Nilsson, Lund*

Från Lunds Universitet, Institutionen för Hygien

**"A method to test the visual and tactile discrimination,
retention, motor coordination and learning abilities
of the Squirrel monkey (Saimiri squireus)"**

**Metod att testa visuell och sensorisk diskrimination, retention,
motorisk koordination och inläring hos Dödskalleapa
(Saimiri squireus) ***

*av Fil. kand. Jan Hellberg, fil. dr. Mats Nyström och
instrumentmakare Olle Nilsson, Lund*

Från Lunds Universitet, Institutionen för Hygien

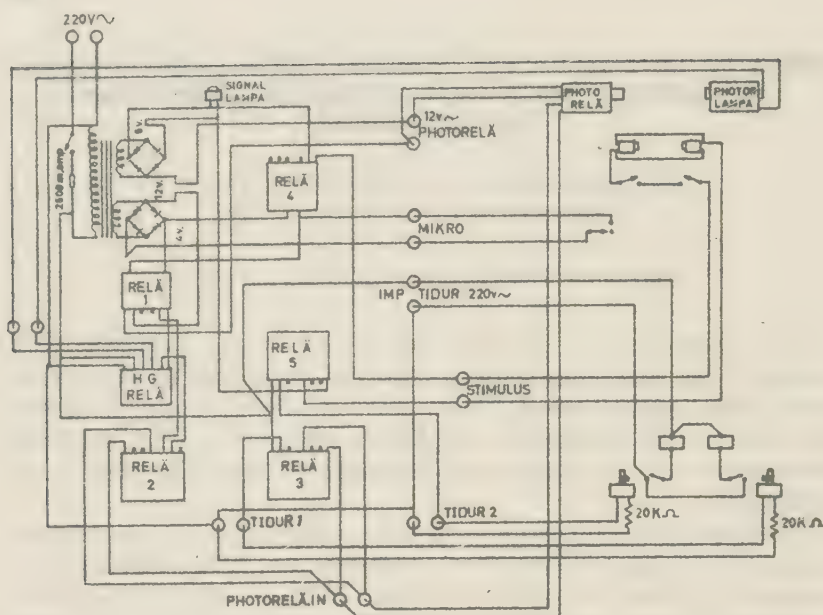
Vid undersökningar som syftar till att utröna vilka verkningar olika toxiska ämnen har på den levande organismen, är det önskvärt att på något sätt erhålla objektiva värden på de förändringar som eventuellt inträffar. En metod är att konstruera ett test eller ett batteri av test, som används på försöksdjuret eller försökspersonen under den tid exponeringen för ämnet ifråga pågår.

Vid Institutionen för Hygien i Lund pågår för närvarande en undersökning av hur organiskt kvicksilver, metylkvicksilver MeHg, påverkar Dödskalleapor (Saimiri squireus). Kvicksilvret administreras oralt genom intag tillsammans med födan. Man vet, genom de erfarenheter man fick i Japan i samband med en omfattande förgiftningsolycka, att hos människor påverkas först och främst det centrala nervsystemet, CNS, med uttalade symptom på koordinations- och synförmåga. Sensitiviteten i fingrarna påverkas också på ett tidigt stadium. Det finns anledning att förmoda att en apa påverkas på liknande sätt. En testmetod som skall ge objektiva värden på förändringar förorsakade av kvicksilverförgiftningen, bör om möjligt innehålla moment som tvingar apan att använda synen, känseln, motoriken på något sätt samt någon relativt välspecificerad högre mental funktion. Det senare för att om möjligt upptäcka eventuella skador på sådana funktioner, något som kan förmodas inträffa mot bakgrund av att skadorna drabbar CNS i första hand.

Mot bakgrund av ovanstående har en metod utarbetats enligt följande: En testapparat, som kan appliceras på fronten av den bur där försöksdjuret förvaras, används för att presentera diskriminationsproblem, parvisa stimuli vid visuell diskrimination och flera stimuli vid sensorisk. Oberoende av vilka stimuli som används mäter apparaturen latens-tid, reaktions-tid, eller med andra ord den tid det tar för apan att göra sitt val, samt den tid det tar för apan att med hjälp av en kedja draga stimulus inom räck-

* Föredrag hållet vid Föreningens för omgivningshygien sammanträde den 28 november 1970 i samband med den medicinska riksstämman.

Bild 1



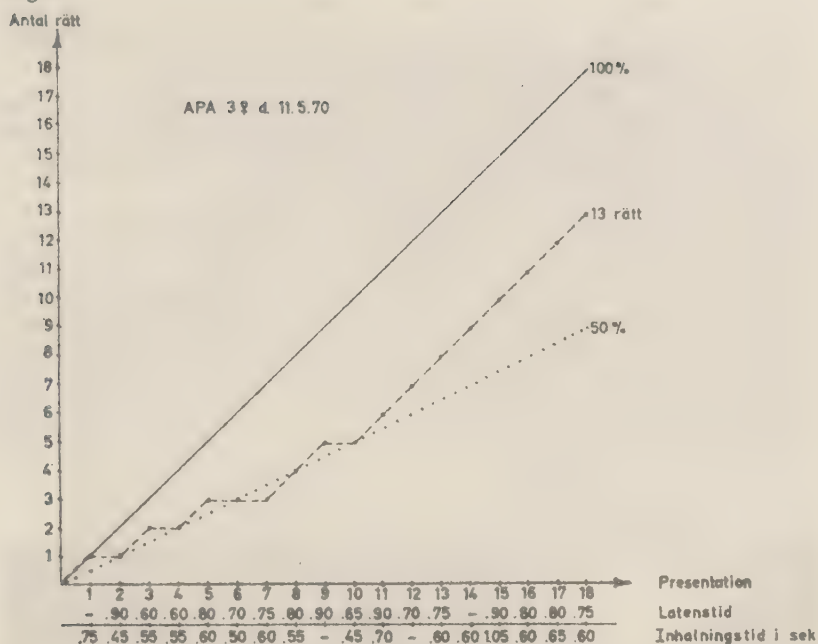
Kopplingsschema över tidmättningsapparaturen. (instrumentmakare Olle Nilsson)

håll. Genom att, då parvisa stimuli används, ett nytt stimulipar presenteras vid varje testtillfälle, kan den inlärningsprocess som innebär att apan lär sig vilket stimulus som är rätt studeras. Det är även möjligt att undersöka apornas retentionsförmåga, minnesförmåga, med apparaturen.

Denna är en modifiering av den så kallade WGTA (Wisconsin General Test Apparatus), vilken i olika modifierade former används vid en mängd försök där stimuli skall presenteras i successiva valsituationer. I den aktuella modellen är tidtagningen en viktig komponent. Den höj- och sänkbara luckan påverkar en mikrokontakt, och startar ett tidur vid början av den uppåtgående rörelsen. Då apan sträcker sig efter valt stimulus, bryter den en ljusstråle och ett fotocellrelä stannar tiduret. Denna tid kallas latentstid. Apan förvarnas omedelbart innan luckan går upp, med en ljudsignal i form av en knackning, och sitter då i de flesta fall beredd. Vår erfarenhet är att få tider måst strykas ur protokollet därför att apan har haft annat för sig. Då apan börjar dra i den kedja som är fäst vid "släden" med stimulus, startar ett andra tidur. Släden påverkar en mikrokontakt, och när kedjan sträcks bryts kontakten och det andra tiduret stannar. Denna tid kallas inhalningstiden.

Bilderna 1—4 visar systemet för de olika tidtagningarna, och kopplings-

Figur 1



Kurvan visar hur de 13 rätta responserna fördelat sig över de 18 presentationerna av diskriminationsproblemet. Lägg märke till hur apan vid presentation 10 lärt sig vilket stimulus som är det rätta. Latens- och inhalningstider står angivna vid respektive presentation.

schemat hur kretsarna är uppbyggda, bl. a. den viktiga krets som möjliggör att ljuskällan släcks och förblir släckt när ljusstrålen bryts av apan. När luckan sänks ned tänds ljuskällan igen, och apparaturen är automatiskt iordningställd för nästa presentation. Figuren visar resultatet av en testning, där den kumulativa kurvan vid presentation 10 och framåt markerar att apan lärt sig vilket stimulus som är rätt. De aktuella latens- och inhalningstiderna är redovisade vid respektive presentationer.

Variationsmöjligheterna vad beträffar tredimensionella stimuli är mycket stora, och det enda som bör beaktas i sammanhanget är att belöningen, t. ex. ett russin, helst skall finnas i omedelbar anslutning till rätt stimulus. Bild 5 visar en modell att lösa detta problem vid prestationer av tvådimensionella stimulus.

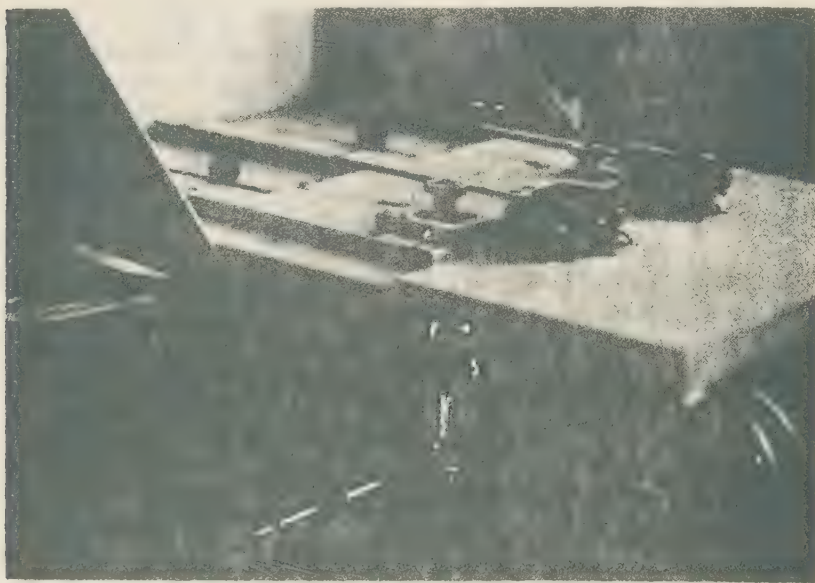
Retensionsförmågan kan testas om man på de båda slädarna fäster en brygga som förbinder dem, och på denna brygga monterar ett antal identiska stimuli. I det läge då stimuliarrangemanget befinner sig utom räckhåll för apan, placeras belöningen i anslutning till något av stimuli medan

den ser på, varefter luckan sänks ned och hålls nere den tid som förutbestämts. Under denna tid förs arrangemanget inom räckhåll, luckan drages upp och apan får välja. (Bild 6).

Apparaturen kan lätt förses med avskärmningsanordningar och en "one-way screen", men vår erfarenhet är att aporna tar mycket liten notis om annat än testproblemet. Undantag är naturligtvis om något extraordinärt inträffar.

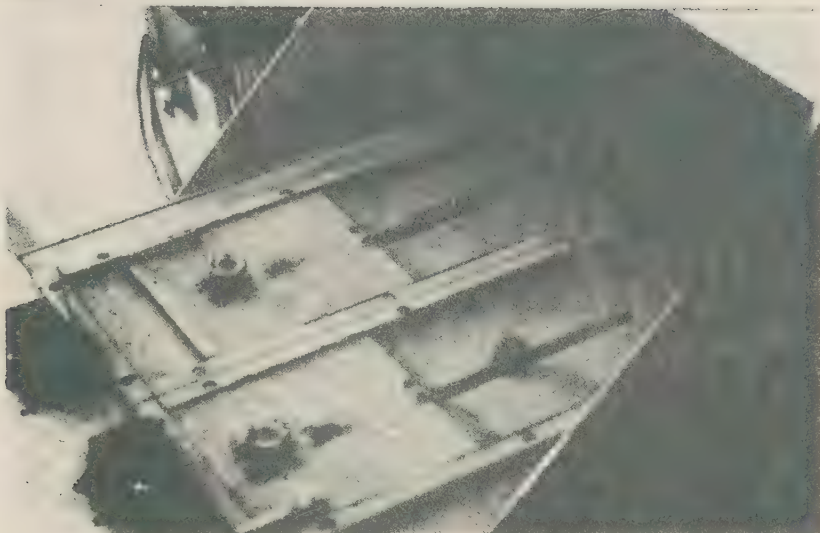
Sammanfattningsvis kan sägas att metoden erbjuder möjligheter att objektivt studera förändringar i latenstider och den speciella motorik som är förbunden med inbälgningsbeteendet. Vidare kan en mängd visuella och sensoriska funktioner studeras genom de rika möjligheter till variationer i stimulihänseende som metoden erbjuder. En naiv apa lär sig apparaten och är beredd att testas på c:a en vecka, vilket tidsmässigt måste anses som gynnsamt. Metoden är tekniskt okomplicerad och kostnadsmässigt mycket överkomlig.

Bild 2



Kedjorna hänger ned genom slitsar i det rågröta bordet och sitter längst ned fast mellan en metallstötta och en mikrokontakt. När apan tar tag i den lilla kulan och börjar dra lossnar kedjan och en strömkrets slutes och tidtagningen börjar. När så mycket av kedjan är inbälad att släde börjar röra sig, brytes strömmen genom de båda mikrokontakter som påverkas av slädarna och som synes längst till höger på bilden.

Bild 3



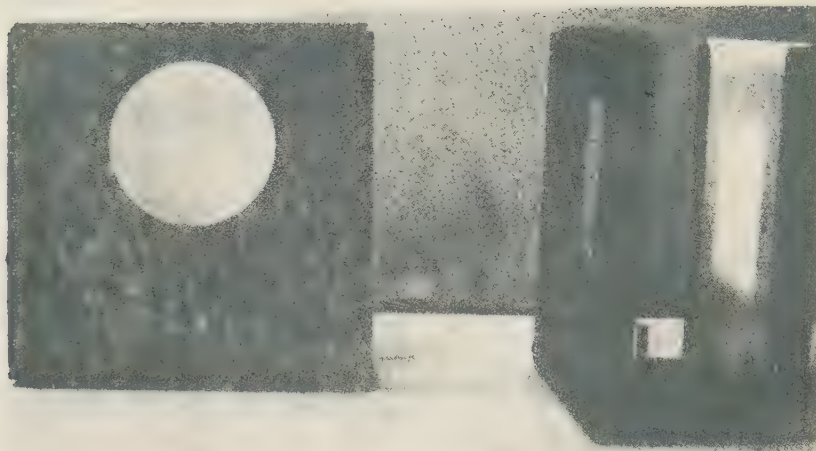
Bilden visar de båda rörliga plattformarna där stimuli fästes med skruvarna. Den ena kedjan hänger ned släsen, medan den andra är sträckt och släden har åkt fram så långt att den slutat påverka mikrokontakten längst till vänster på bilden. Fotocellutrustningen sitter på ömse sidor om öppningen, och i det cirkelrunda hålet syns avbländningsanordningen, som gör att den verksamma ljusstrålen har samma diameter som det lilla hålet.

Bild 4



Tiduren för latenstider och inbathingstider samt kopplingsboxen för de elektriska komponenterna. Räkneverken ovanför urtavlorna möjliggör mätningar av tider över två sekunder.

Bild 5



Anordning för att presentera tråddimensionella stimuli. Den lilla tagg som syns mellan främre och bakre plattorna används för att fästa belöningen, ett russin, på. Apan sträcker alltså in handen genom figuren för att nå belöningen. Längst till höger skymtar den metallunga som används för att fästa stimulus vid den rörliga släden. Genom att reglera färg- eller nyansförhållandet mellan främre och bakre platta kan man nå mycket stor variation i kontrast mellan aktuell figur och bakgrund.

Bild 6



Arrangemang för att testa vetentionsförmågan. De fyra klossarna är identiska till utseende och form och fästa med gängjärn i bakkanten. En urgröpning i botten möjliggör att belöningen kan placeras på "bryggan" och sedan döljas av klossen då denna fälls ned. På bilden finnes inget arrangemang för att mäta inbaltningstiden, men det är mycket lätt att komplettera med ett sådant. Notera att alla fyra klossarna rör sig samtidigt då bryggan skjutes eller drages in mot apan.

Bild 7



Dödskalappa (Saimiri sciureus). Arten förekommer vild i Sydamerika och Centralamerika. Stora mängder fångas och exporteras främst till USA där den är ett populärt föröksdjur främst p. g. a. sin relativt höga intelligens och välutvecklade hjärna. Apan är liten och lätthanterlig och mycket lite aggressiv, medelvikten ligger omkring 600 gram.

Summary

"A method to test the visual and tactile discrimination, retention, motor coordination and learning abilities of the Squirrel monkey (Saimiri sciureus)"

When studying the effects of toxic substances on the living organism, it is valuable to use methods that give objective and replicable data. Methyl mercury given orally affects the central nervous system, causing changes in visual and other perceptual abilities, motor coordination, and balance. At the Department of Hygiene, University of Lund, Sweden, squirrel monkeys are being fed methyl mercury in small lumps of semi-hard gruel. In an attempt to objectively evaluate changes in vision, learning, retention, and motor coordination, visual discrimination problems have been presented

in a modified Wisconsin General Test Apparatus (WGTA) which can be mounted on the front of individual living cages. Two stimuli, selected from a group of geometrical solids, are placed on sliding platforms out of the monkey's reach. To obtain the reward under the "correct" stimulus, the monkey must reel in the platform by means of an attached string. Two different response latencies are recorded automatically on each trial. The first latency is the time it takes the monkey to make a choice and reach for one string or the other. The second latency, which is used as a measure of motor coordination, is the time required to pull in the platform. Since a new stimulus pair is presented every day, the monkey's ability to form a "learning set" over a period of time is also tested.

Litteratur:

- Johnson, J. I. och Michels, K. M., Discrimination of small intervals and objects by raccons, *Animal Behavior*, VI, 3—4.
- Peterson, M. E. and Rumbaugh, D. M., Role of object-contact cues in learning-set formation in Squirrel monkeys, *Perceptual and Motor Skills* 1963, 16, 3—9.
- Rosenblum, L. and Cooper, R., *The Squirrel Monkey*. Academic Press 1968.
- Woodburne, L. S., Visual Acuity of "Saimiri sciureus", *Psychon. Sci.*, 1965, 3, 307—308.

PSYCHOLOGICAL RESEARCH BULLETIN

XII:8 1972

*The Influence of Methyl Mercury Exposure on
Learning-Set Behavior of Squirrel Monkeys
(Saimiri sciureus)*

by Jan Hellberg and Mats Nyström



LUND UNIVERSITY SWEDEN

A b s t r a c t. - Measurements of reaction time and learning-set performance in ten squirrel monkeys were obtained four times a week in a modified WGTA apparatus. Half of the subjects were given either subacute or prolonged intoxication with methyl mercury, the other half serving as controls. The impairment in the subacute group was sudden blindness, rendering further testing impossible. Prolonged exposure resulted in the gradual development of the symptoms of ataxia, defective motor coordination and restricted peripheral vision over the period of a week. Symptoms were correlated with increasing reaction times, but learning-set as such did not deteriorate. Thus, prolonged exposure gave rise to symptoms similar to those found in man.

Methyl mercury (MeHg) given to higher organisms in food accumulates in the CNS, especially in the visual system. Intoxication causes severe visual impairments, and finally total blindness and death (e.g. Nordberg, Berlin & Grant, 1971). Intoxication of man and animals due to MeHg exposure through the abundant use of insecticides and the accumulation of various forms of waste products in fish are now regrettably well-known and prevalent in many parts of the world.

The present report¹ is part of a larger project concerned with morphological and behavioral changes associated with this form of poisoning. The specific aim here is to investigate whether signs of behavioral deviations can be detected and be temporally related to the body burden of MeHg as measured by its blood concentration, as well as to morphological changes. A modified WGTA apparatus has been developed in order to obtain cumulative and standardized measures of behavior in successive discrimination tasks (Hellberg, Nyström & Nilsson, 1971).

1) This study was partly supported by grants from The Swedish Council for Social Science Research

S u b j e c t s

Three adult male and two adult female squirrel monkeys (*Saimiri sciureus*) who were exposed to MeHg constituted the experimental group. They had five counterparts in a control group, possessing a level of MeHg between 10 and 15 ng per ml blood.

P r o c e d u r e

Behavioral testing. Prior to mercury exposure all animals were placed in individual 46 x 37 x 45 cm cages for a week of pretraining in the test apparatus. Subsequently, poisoning of the experimental animals, who were selected at random, was started, both these and the control animals being tested four times a week using two choice alternatives, one of which was regularly rewarded. The task for the monkey was to pick up one of the two chains (each 20 cm long) and to haul in a corresponding sled with the stimulus attached. The stimulus pairs were arranged in such a way that the two stimuli always differed in form and color as well as size. The "correct" stimulus within the pair (it contained a hidden raisin) was first determined by chance, its right-left position subsequently being randomized within the test series of 18 presentations, which were separated by 20-second pauses. One series was administered each test day using a new stimulus pair each day. The same stimulus pair was employed for all subjects on any given test day, the order between the pairs employed being varied over days according to a random sequence as was the order of testing the different subjects on a given day.

Reaction time and choice, as well as the monkey's behavior in the choice situation, were recorded. In addition, some films were taken in order to document the types of behavior which were characteristic, especially after the onset of

clinical symptoms. The data were analyzed for the number of correct choices (R), the mean latency time for selecting the chain (ML), and the mean time required for pulling in the stimulus (MI) in each test session.

Mercury exposure. MeHg was mixed in with ordinary child gruel, formed into 1 g lumps containing about 0.6 mg mercury each. Lumps were offered the experimental animals at a rate calculated to produce a toxic state within one month (subacute condition) or two months (prolonged condition) respectively. Subsequently, a dose which simply counterbalanced the natural excretion of the poison was given. Regular controls of the mercury level were obtained using blood samples that were analyzed by means of a combined chemical and atomic-absorption method (Schütz, 1969).

R e s u l t s

The latent period between attainment of the toxic level (1200 ng Hg/ml blood) and development of symptoms differed between the subacute and the prolonged conditions (Fig. 1). In the former group, complete symptoms developed within a few days after the first clinical signs, making further testing impossible because of blindness. The prolonged condition, on the other hand, resulted in the gradual development of signs which could be detected concurrently in the test scores. However, even during the latent period there is a trend toward greater variability in the test scores of all of the experimental animals as compared with their controls, only one of the 15 standard deviations (three behavior measures x five subject pairs) being greater in the control subject ($P < 0.01$, sign test). The mean levels of the three measures appear to be stable up to the occurrence of the first symptom (Fig. 2), but then latency and duration increase in the

experimental group, i.e. in the prolonged subgroup, the one where the data still could be secured. Fig. 3 shows the difference in test scores between the experimental and control animals in this subgroup during the last three weeks of testing. Significant correlations between these differences and consecutive test sessions further indicate the increase in duration and latency in the experimental animals. The number of correct choices remains above the random choice criterion in most cases.

D i s c u s s i o n

The only effect observed during latent intoxication is a general increase in the variability of the learning-set behavior. Specific effects in the form of longer reaction times for selecting and pulling in the stimulus do not appear prior to the symptom, i.e. to the point in time when ocular observation and films reveal the monkey to have difficulties presumably caused by constriction of its visual field. Therefore, reaction time seems insensitive to latent MeHg intoxication, although clear effects could be observed in the acute state. The monkeys remained motivated to perform in spite of their handicaps and the learning set per se did not deteriorate.

It was shown in an earlier report (Berlin, Nordberg & Hellberg, 1971) that the content of MeHg in the body as well as the head is linearly related to the cumulative blood content of MeHg. At the same time, the concentration of mercury in the head was found to be concentrated subcortically in the cerebrum, particularly in the occipital region. The present findings indicate that the impairment after subacute exposure results in blindness, whereas prolonged exposure affects vision gradually. The long reaction times in the latter case coincide with an observable disturbance in the fi-

ne movements of the fingers and in visual-spatial coordination. These monkeys had obvious difficulties in locating a fixed object in space and in grasping it after it was found. In conclusion, the symptomatology for the animal given prolonged exposure corresponds to that reported for man (Berglund et al., 1971). Brain injury appears to be limited to the visual cortex, the primary sensory and the motor areas, while higher functions are unaffected.

We are now developing a more sensitive behavioral instrument to detect latent MeHg intoxication at an earlier stage, making use of the finding that peripheral vision is impaired first.

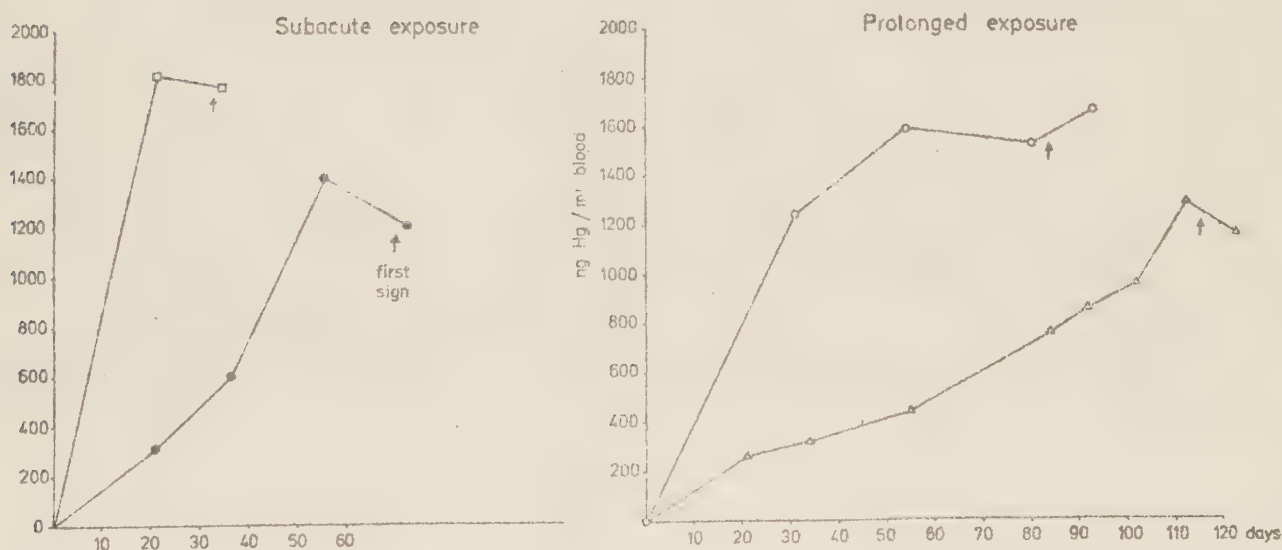
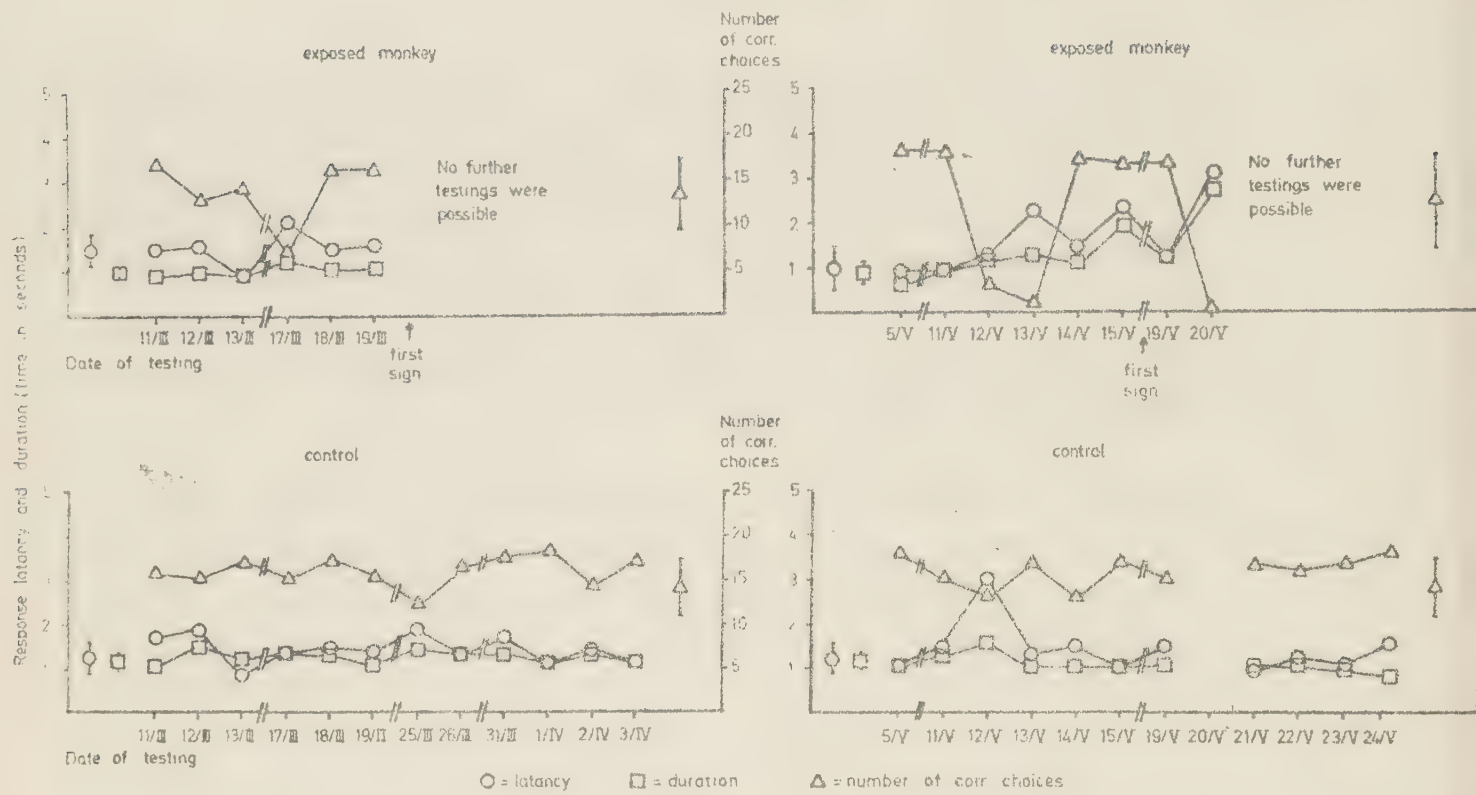


Figure 1. Temporal blood concentration in two monkeys with subacute and prolonged exposure to MeHg. (The data of the third individual in the second group are missing due to analytical error.) Arrows indicate the onset of obvious clinical signs.

Subacute exposure



Prolonged exposure

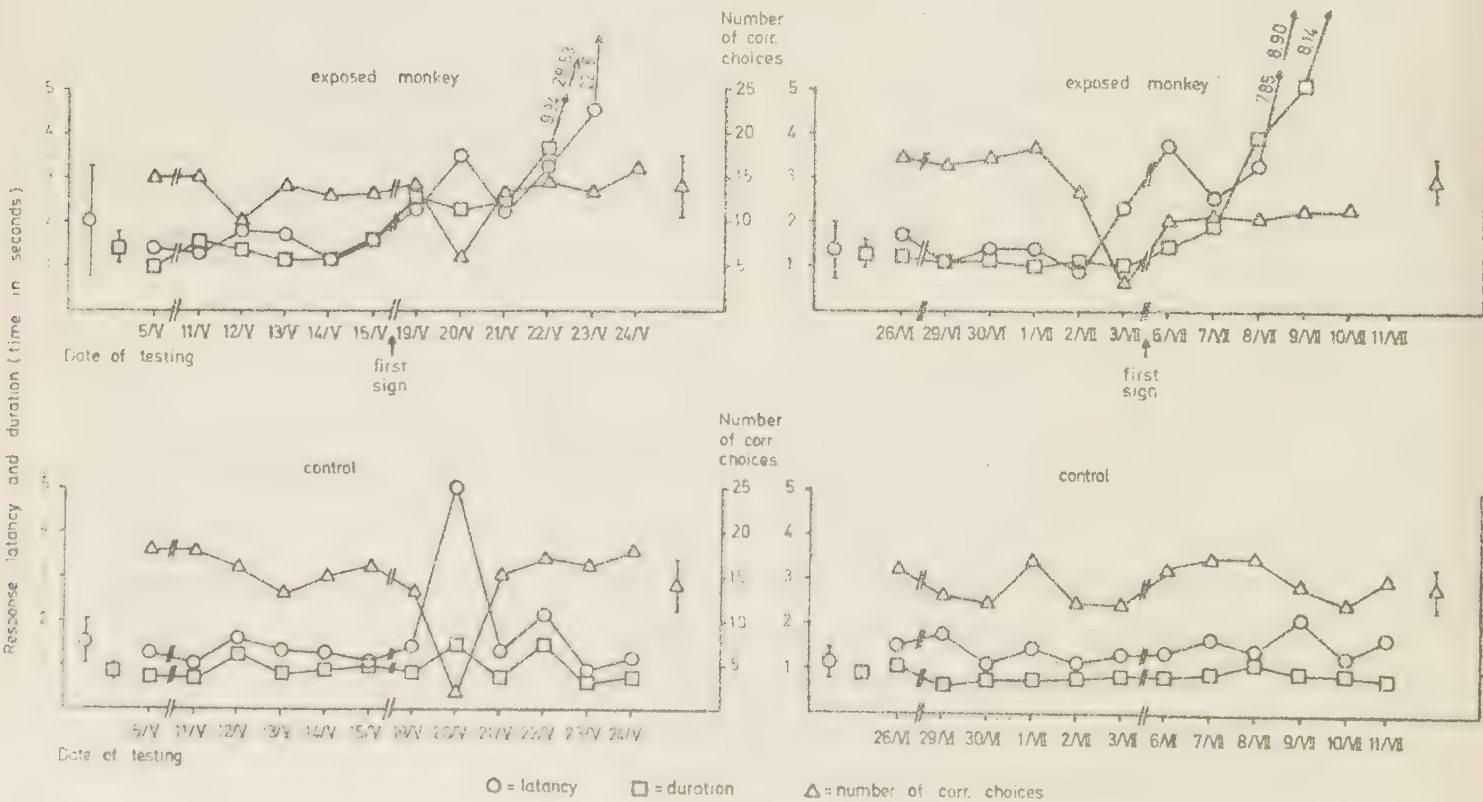


Figure 2. See next page.

Prolonged exposure

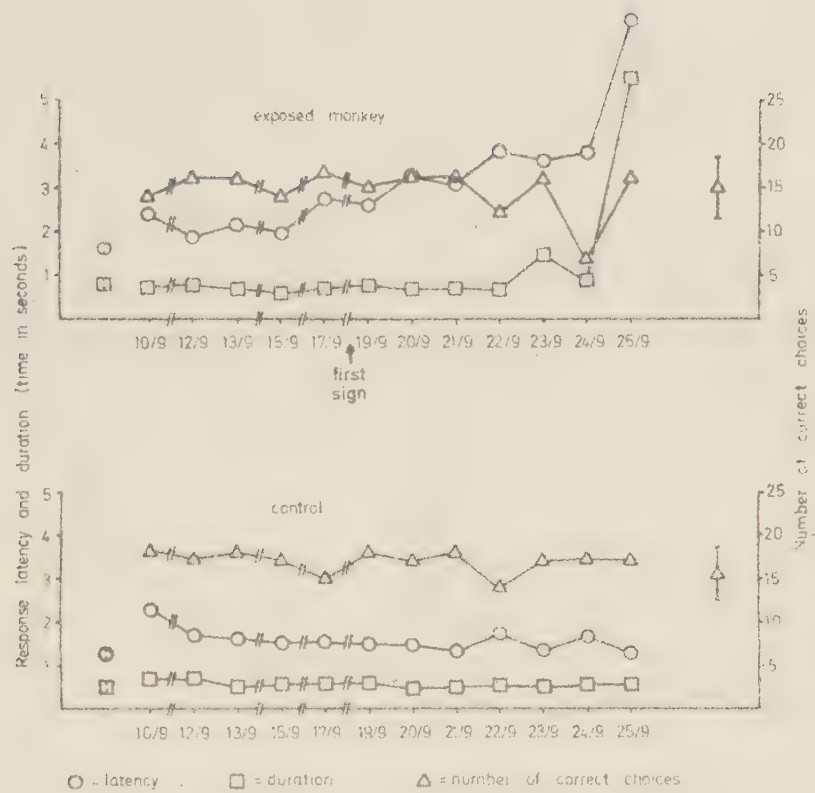


Figure 2. Daily mean scores on three measures of learning-set performance for five pairs of experimental and control monkeys some days before and after the onset of signs. Mean and standard deviations of the whole presign period are indicated next to the relevant axis.

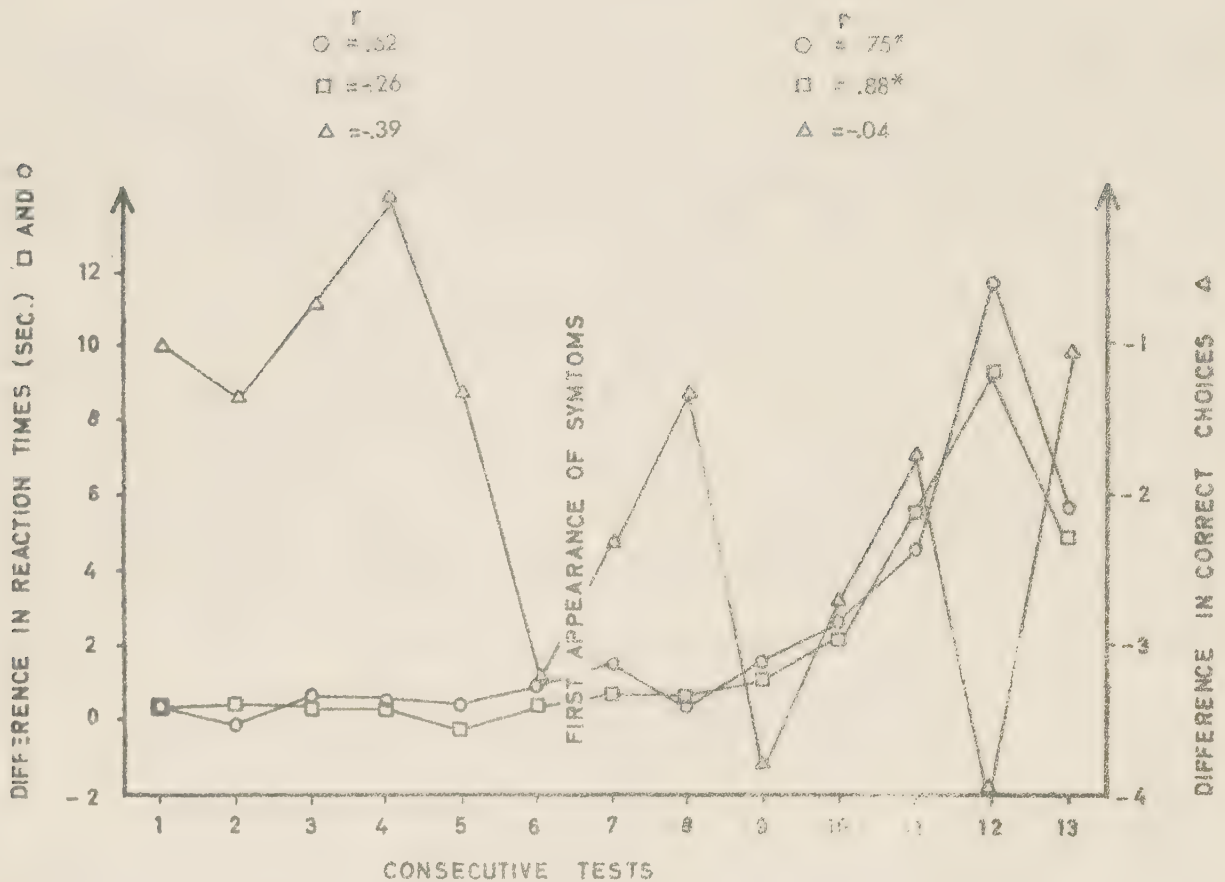


Figure 3. Mean differences in performance between experimental and control monkeys on the test sessions before and after the onset of symptoms. Correlation coefficients between performance and the order of consecutive sessions within the respective periods are also indicated.

R e f e r e n c e s

- Berglund, F. et al. (1971). Methyl mercury in fish. A toxicologic-epidemiologic evaluation of risks. (Report from an expert group). Nord. Hyg. Tidskr., Suppl. 4. Pp. 5-364.
- Berlin, M., Nordberg, G. & Hellberg, J. (1971). The uptake and distribution of methyl mercury in the brain of *Saimiri sciureus* in relation to behavioral and morphological changes. (Mimeogr. Inst. Hygien, Lund Univ., Sweden).
- Hellberg, J., Nyström, M. & Nilsson, O. (1971). Metod att testa visuell och sensorisk diskrimination, retention, motorisk koordination och inlärning hos Döds-
skalleapa (*Saimiri sciureus*). Nord. Hyg. Tidskr., LII, 14-21.
- Nordberg, G., Berlin, M. & Grant, C.A. (1970). Methyl mercury in the monkey - autoradiographical distribution and neurotoxicity. Proc. 16th Int. Congr. Occup. Health. Tokyo. Pp. 234-237.
- Schütz, A. (1969). Metod för bestämning av små mängder kvicksilver i blod, urin och annat biologiskt material. Occupat. Med. Klin. Report 691020. Lund Univ., Sweden.

PSYCHOLOGICAL RESEARCH BULLETIN

XVII:12 1977

*Changes in Scotopic Flicker Fusion Intensity (cffi)
in Squirrel Monkeys Exposed to Methyl Mercury*

by Jan Hellberg and Jonas Hellström



LUND UNIVERSITY SWEDEN

A b s t r a c t .¹ - Tested critical flicker fusion intensity (cffi) in seven Squirrel monkeys (*Saimiri sciureus*) orally exposed to Methyl Mercury (MeHg), until clinical signs developed. Flicker rate was held constant and intensity was systematically varied within the thresholds for scotopic (dark) vision. The results showed that an increase in cffi correlated to toxic blood levels of MeHg. In an early stage these increases were reversible. Later on the increase became irreversible but appeared well in advance of conventional clinical signs.

When clinical signs² appear in Squirrel monkeys (*Saimiri sciureus*) that have been given Methyl Mercury (MeHg) orally, an irreversible stage of poisoning is reached. The signs consisting initially of visual impairment and ataxy of the fingers, are caused by lesions in cortical tissue, and parallel those described in man (Berlin, Nordberg and Hellberg, 1973; Grant, 1973).

Attempts have been made in our laboratory to trace effects of the lesions during the early stages before clinical signs appear. The method used was based on a modified WGTA (Wisconsin General Test Apparatus) and required that monkeys discriminated visually between two stimuli and pulled the chosen stimulus within reach by means of a chain, thus obtaining a reward. Latency, time for pulling and number of prestations before reaching a 100% correct choice criterion were noted. Results showed none of these functions to be affected prior to the appearance of conventional signs (Hellberg and Nyström, 1972).

The present investigation is a further attempt to trace MeHg lesions in monkeys before clinical signs appear. Since the visual impairments are the earliest and most pronounced signs, a

¹ This work was supported by a grant from the Swedish Medical Research Council (Project No. 2081).

² In animal research "clinical signs" denote all types of differential diagnostic information that can be detected by direct observation.

method was sought for detecting these impairments prior to the appearance of clinical signs. Phenomenologically the latter can be described as a gradual, concentric constriction of the peripheral visual field (Methyl Mercury in Fish, 1971).

Peripheral portions of the monkey retina contain very few cones but are densely packed with rods. Thus, they serve as the basis for scotopic (or night) vision, just as in man.

Since the early visual impairments following exposure to MeHg consist of constriction of the peripheral visual field, it was assumed that a test procedure aimed at tracing cortical lesions in a pre-clinical stage should be based on sensory thresholds for scotopic vision.

De Valois (1965) describes a method for revealing the change from photopic to scotopic vision in primates. The method is based on critical flicker fusion intensity (cffl) measurements, the flicker rate being held constant at 10 cps and the light intensity being systematically varied. The results obtained by de Valois revealed intensity levels for man and Squirrel monkey to be identical.

Thus, for determining cffi in Squirrel monkeys and employing a constant flicker rate an apparatus was constructed according to the above principles. It was hypothesized that an increase in cffi would occur among monkeys exposed to MeHg. It was also assumed that the predicted changes would appear prior to conventional clinical signs.

M e t h o d

Apparatus

During testing the monkey was kept in a small aluminium cage. This was also employed for transporting the monkey between the living cage and the test situation. The cage had two mov-

able gables, one of transparent plexiglass and the other of wallboard. When testing took place, the wallboard gable was replaced by a panel that contained two square stimulus windows (5.5×6 cm) of opaque plexiglass and a pellet dispenser which fed pellets into a small cup situated between and slightly below the windows. Thick black paper with a circular hole ($\varnothing$ 3 cm) covered each window. Circles of light slightly larger in diameter than the holes, were projected on the outside of the windows. One light was steady whereas the other flickered at a rate of 10 cps. Both lights were of equal intensity. Their relative positions could be interchanged at any time. If the monkey pressed the window with flickering light, a reward was automatically fed into the cup and both lights were turned off. Pushing on the window which was steadily lit only caused the lights to be turned off (Figure 1).

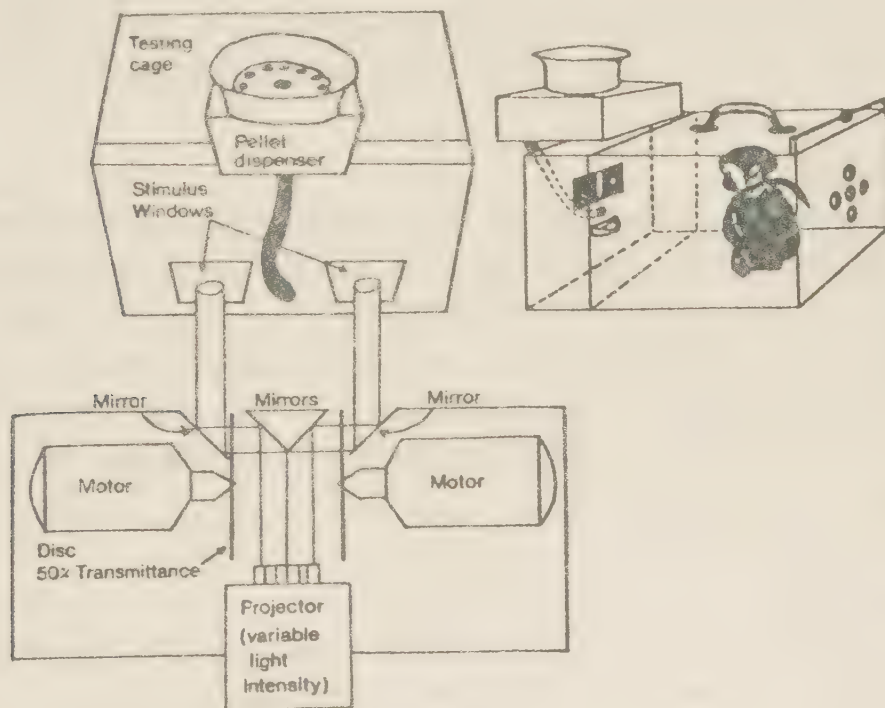


Figure 1. Diagram of the test apparatus. See text for a detailed description.

Flicker rate was held constant but light-intensity could be varied from 0 to 8 lux, measured from inside the cage. To allow for changes of light intensity, a projector was connected to a rheostat. The beam was split into two identical parts by means of mirrors and then projected on the two stimulus-windows.

The flicker was produced by a rotating disc (10 Hz), half of which was painted black, the other half being transparent. To compensate for the filter effect caused by the disc, a similar disc was inserted in the beam for steady light and was made to rotate fast enough to produce flicker well above cff (critical flicker frequency). By interchanging the voltage between the two motors driving the discs, the relative positions of the steady and the flickering lights could likewise be interchanged. Photo-electrical cells were placed in each beam to allow for continuous checks of equal intensity. Furthermore the preset speeds of the motors were checked at intervals by means of a stroboscope.

Experiments

Two experiments were carried out. Experiment I involved first training the monkey in use of the apparatus and establishing of normal cffi, followed by exposure to MeHg and continued testing until clinical signs appeared. Experiment II employed the same procedure as in Experiment I, but the tests were performed in another room with a somewhat higher background intensity.

Subjects

In Experiment I the subjects were 2 adult Squirrel monkeys (1 male and 1 female) in the experimental group and two others (likewise male and female) in the control group. When the animals in the experimental group had developed clinical signs, the male control animal was also exposed and tested until signs appeared. In Experiment II the subjects were 4

adult Squirrel monkeys, all of which were exposed to MeHg and served as their own controls.

Training

Training consisted of shaping by means of successive approximations in which the monkeys was trained first to go into cage willingly, and then to accept being transported to the room where testing took place.

To train the monkey to react to the flickering light, the steady light was first covered up. These conditions were maintained until the monkey responded to the flickering light by pressing on the window. When this was achieved, both lights were presented simultaneously, their relative positions being interchanged according to a random sequence. Light intensity was kept at the maximum.

When the monkey responded correctly to the flickering light, the tests for cffi took place.

Testing

In the first experiment a successive lowering of the light intensity and a continual random shift of the steady and of the flickering light between right and left continued as long as the monkey responded correctly. A correct response was defined as at least 75% correct choices of the flickering light during 20 presentations at a given intensity. When this criterion was not met, a one-step increase in light intensity was made and discrimination at the new level then being tested. Cffi was defined as the lowest intensity level at which 75% criterion was met.

In the second experiment a successive lowering of light intensity and a continual random shift of the steady and the flickering light from left to right was continued until a

wrong response occurred. When this happened a one-step increase in intensity was made. If this led to a correct response, the intensity was then lowered by one step. This procedure was continued until a threshold, expressed as the lowest intensity at which the monkey could discriminate between the steady and the flickering light, was established. The inside of the cage was semi-dark. The testing room was dimly lit by a shaded lamp. The individual animal was tested once a day 5 - 7 times a week.

MeHg-exposure

A weekly dose of mercury, in a solution containing 0.4 mg MeHg/ml, was given through a plastic tube inserted into the ventricle. Blood samples were taken 4 days after exposure, through a vein in the tail or at the back of the knee. Controls (in the first experiment) were fed water by the same procedure and blood samples was taken as well. The amount of MeHg administered was calculated so as to produce a toxic blood level of MeHg (1200 ng Hg/ml blood) within three weeks and to then maintain this level until clinical signs developed (Berlin, Nordberg and Helberg, 1973). This was achieved by a weekly dose of 0.4 mg MeHg/kg body weight during three consecutive weeks, followed by a weekly dose of 0.2 mg MeHg/kg body weight.

Testing and exposure continued until clinical signs developed and became so pronounced as to hinder the monkey from discriminating between the two lights of highest intensity level.

R e s u l t s

Experiment I

The mean number of sessions for training the monkeys to respond correctly at the highest intensity level was 13.5. This figure does not include the shaping procedure.

Individual variation in the number of sessions required to reach the discrimination criterion at the highest intensity level was considerable. The shortest period was 9 sessions and the longest 17 sessions.

Figure 2 shows that cffi differs very little between individuals, the mean being .08 lux. There is a radical drop from 85% correct responses at .3 lux to about 50% at .01 lux. The overall response rate is still high at the last intensity but below .01 lux there are very few responses.

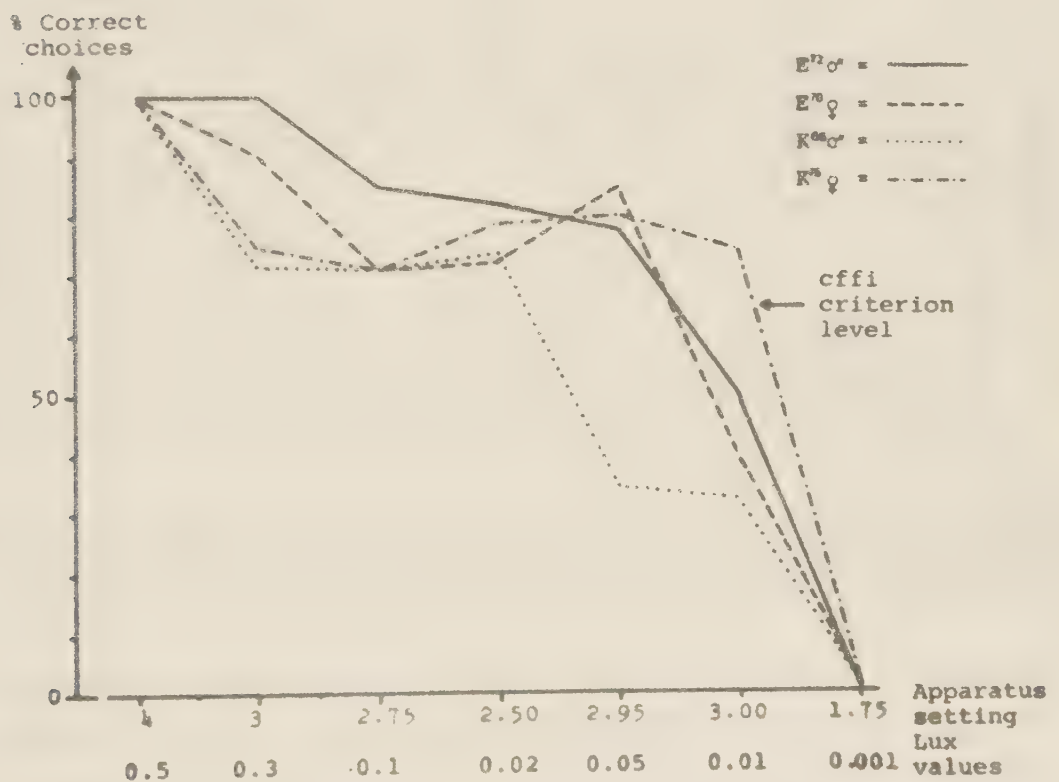


Figure 2. Cffi for the four monkeys in Experiment I before the exposure to MeHg commenced.

Two important facts are evident from Figure 3 (showing the development after exposure commenced): (1) In at least two cases the increase in cffi is only temporary (reversible) and is correlated with a temporary rise in MeHg-blood level to well above toxic level (cf special marks in Figure 4); (2) the increases in cffi, both of the reversible and the irreversible type, appear a considerable time before any of the clinical signs.

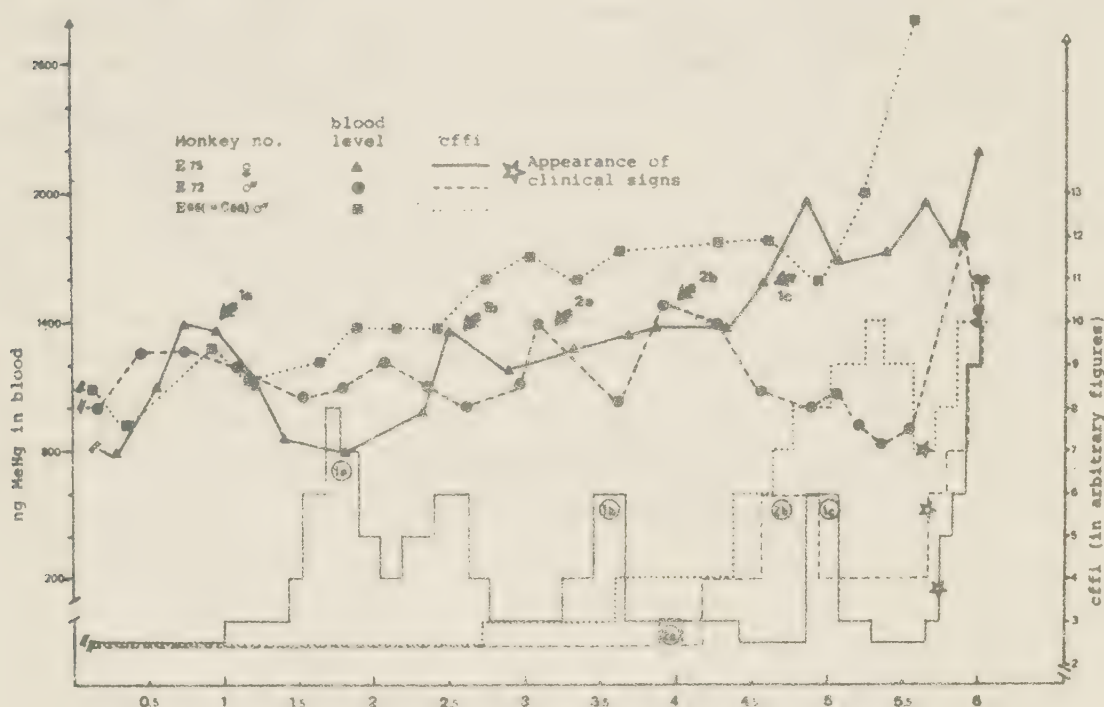


Figure 3. Cffi and MeHg-blood level for the three exposed monkeys in Experiment I. Five temporary rises in blood levels and the corresponding increases in cffi are specially marked (1a, 1b, 1c, 2a and 2b). The appearance of clinical signs is specially marked.

There is a significant correlation between the increase in cffi and the rise in the MeHg-blood level when calculated a phase shift of six days ($r = 0.76$). Thus, an increase in cffi appears six days after administration of a dose sufficient to raise the blood level.

The third monkey, which first served as control and then was exposed according to a faster schedule, "K"♂ in Figure 3,

revealed a steady rise in MeHg-blood level without any marked peaks. This resulted in an irreversible increase in cffi, although there is a considerable period of time between the first increase in cffi and the onset of clinical signs.

Experiment II

Figure 4 shows in a diagrammatic way cffi, MeHg-blood level and the changes in these values over time for the four monkeys in this experiment. The onset of clinical signs is specially marked.

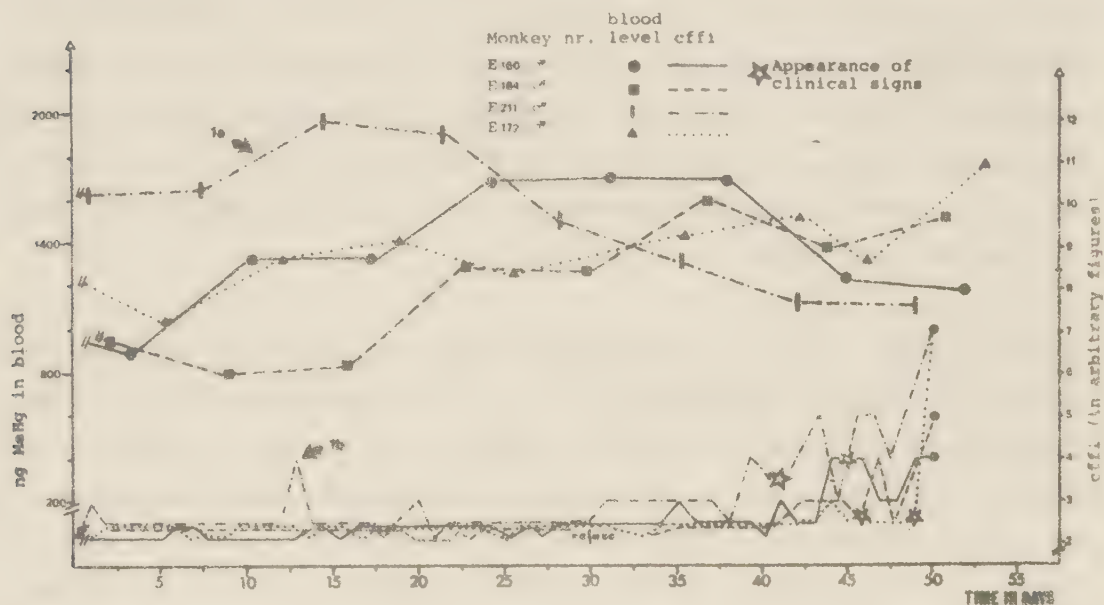


Figure 4. Cffi and MeHg-blood level for the four monkeys in Experiment II. The appearance of clinical signs is specially denoted. In one case a temporary rise in MeHg-blood level and the corresponding increase in cffi is marked (1a and 1b).

Even for these monkeys it is evident that an increase in cffi appears prior to conventional clinical signs. A case of reversible increase in cffi which correlated with a temporary rise in MeHg blood level can be seen in one case.

Discussion

The apparatus, the training procedure and the procedure of testing for cffi all proved to be workable here. The monkeys

were easily trained and the apparatus was technically simple and relatively cheap to construct. Once the training was completed, the discrimination tests were easy to administer. However, the training required knowledge of operant conditioning paradigms and some skill and patience from the trainer. This was important since the monkeys differed individually regarding their reaction to training.

The absolute luminance values have not been stressed, since the hypothesis was one of change in cffi in the direction of an increase during exposure to MeHg. The normal levels for cffi were very stable and interindividual differences small. Stability was maintained over very long periods, even when MeHg-exposure and blood sampling were performed parallel to the tests.

Since there are only minor variations in cffi, every increase in cffi to or above .3 lux, even when of short duration, must be considered a reliable sign. An additional reason for assuming that these increases were abnormal is the high cross-correlation with temporary rises in the MeHg-blood level to above 1200 ng Hg/ml blood. Noteworthy, especially when it comes to further work with this method, is the fact that even the irreversible increase in cffi appeared well in advance of any clinical signs.

It has been shown earlier (Hellberg and Nyström, 1972) that the development of clinical signs takes a different course, depending on whether exposure has been acute or subacute, i.e., whether equal total doses have been administered during a short as opposed to a long period. If exposure has been acute, signs develop dramatically and lead to total blindness within 48 hours. This is to be compared with the development following subacute exposure, where signs devel-

op gradually during the course of 6-7 days. The present results support this finding since the increase in cffi never was found to be reversible in the actually exposed monkeys.

Evidently the present method makes it possible to trace cortical lesions in the visual centers of the brain following oral exposure to MeHg. Changes appear prior to any common clinical signs. Thus, the method represents a step towards a clinical method for use in epidemiological surveys of human populations exposed to MeHg.

On the basis of these findings, the next step might be to construct an apparatus for measuring cffi in human scotopic vision. The independent variables could be the same as in this investigation.

It might further be suggested that the apparatus should be mobile, fitted for automatic registration of responses and requiring a minimum of instructions to the person tested. The handling of the apparatus during testing should not require any technical skill from either the subject or the tester.

These requirements are based on the expectation that any epidemiological investigation of MeHg-poisoning will need to be carried out in the field, perhaps by and on individuals having little or no experience with technical equipment.

R e f e r e n c e s

Berlin, M., Nordberg, G. & Hellberg, J. (1973). The Uptake and Distribution of Methyl Mercury in the Brain of Saimiri Sciureus in Relation to Behavioral and Morphological Changes. In M. Miller and T. Clarkson (Eds.), Mercury, Mercurials and Mercaptans. Springfield: Charles C. Thomas.

- Berlin, M., Grant, C.A., Hellberg, J., Hellström, J. & Schütz, A. (1975). Neurotoxicity of Methyl Mercury in Squirrel Monkeys. Arch. Environ. Health, 30, 340-348.
- De Valois, R.L. (1965). Behavioral and Electrophysiological Studies of Primate Vision. In W.D. Neff, (Ed.), Contributions to Sensory Physiology, 1, New York: Acad.Press, Pp. 137-178.
- Grant, C.A. (1973). Pathology of Experimental Methyl Mercury Intoxication: Some Problems of Exposure and Response. In M. Miller and T. Clarkson, (Eds.) Mercury, Mercurials and Mercaptans. Springfield: Charles C.Thomas, Pp. 294-312.
- Hellberg, J. & Nyström, M. (1972). The Influence of Methyl Mercury Exposure on Learning-set Behavior of Squirrel Monkeys (*Saimiri sciureus*). Psychol. Res. Bull., Lund Univ., 12, No. 8.
- Methyl Mercury in Fish: A toxicologic-epidemiologic evaluation of risks. Report from an expert group. Nord. Hyg. Tidskr., Suppl 4, 1971.

PSYCHOLOGICAL RESEARCH BULLETIN

XVIII:1 1978

*A Method for Measurement of Critical Flicker Fusion
Intensity (cffi) in Scotopic Vision*

by Jan Hellberg



LUND UNIVERSITY SWEDEN

A b s t r a c t . - Investigated an apparatus for semiautomatic testing of the level of dark adaptation and Critical Flicker Fusion Intensity (cfffi) in two slightly different ways when flicker was held constant at 10 cps. The aim was to establish a standardized test procedure and normative values which would allow the apparatus to be used as a diagnostic instrument in epidemiological investigations of populations exposed to methyl mercury. A sample from a non-exposed population was tested on three different occasions by three different testers working parallel with three subgroups. The results gave reason to suggest a standardized procedure simpler than the one used in the investigation. Reliability was found to be sufficient. Further investigations should concern the problem of what effects fatigue might have and what can be gained by changing the wavelenth of the stimulus light towards the optimum for rod sensitivity.

It has been shown that Critical Flicker Fusion Intensity (cfffi) in scotopic vision shows an increase in squirrel monkeys (*Saimiri sciureus*) exposed to methyl mercury (MeHg). The lesions caused by the mercury are located in the visual cortex. The increase in cfffi appeared prior to conventional clinical signs of poisoning (Hellberg and Hellström, 1977; Berlin, Grant, Hellberg, Hellström and Schütz, 1975). The method used in these investigations for measuring cfffi was based on discrimination between a steady and a flickering light of equal intensities. Flicker frequency was held constant at 10 cps and the intensity was systematically varied at intensities below the upper threshold for scotopic vision.

Flicker fusion, whether it be dependent on frequency or intensity, is one method which has been used to diagnose brain lesions in man. It has been used to measure the field of vision (perimetry). However, the intensities used have been above the threshold of the cones, i.e. these investigations have concerned photopic vision (Chandler, Parsons and Haase, 1964; Parsons and Huse, 1958; Parsons and Gottlieb, 1960).

In man as well as in squirrel monkeys an early and reliable clinical sign following MeHg exposure above toxic levels, is a successive and concentric restriction of the peripheral visual field (Berlin et al., 1975). Furthermore, we know that the monkey retina as well as the retina of man is densely packed with rods in its peripheral region. These cells are active in scotopic vision (Rosenblum and Cooper (Eds.), 1968).

The above facts were the main reason why sensory threshold for scotopic vision were used in the investigation mentioned earlier (Hellberg and Hellström, 1977). This investigation showed that it was possible to detect early, preclinical signs in that the cffi showed an increase. It is thought that an apparatus constructed according to the same principles can be used as a diagnostic instrument in epidemiological investigations on human populations exposed orally to MeHg. The importance of being able to trace preclinical signs is evident since a majority of the clinical signs are irreversible.

The present investigation had the following goals: To examine an apparatus constructed for the measurement of the level of dark adaptation and cffi in two slightly different ways, to standardize a test procedure and to establish normative values by testing samples from a population not exposed to MeHg. The statistical analysis should be directed towards the question of the degree of stability of the method.

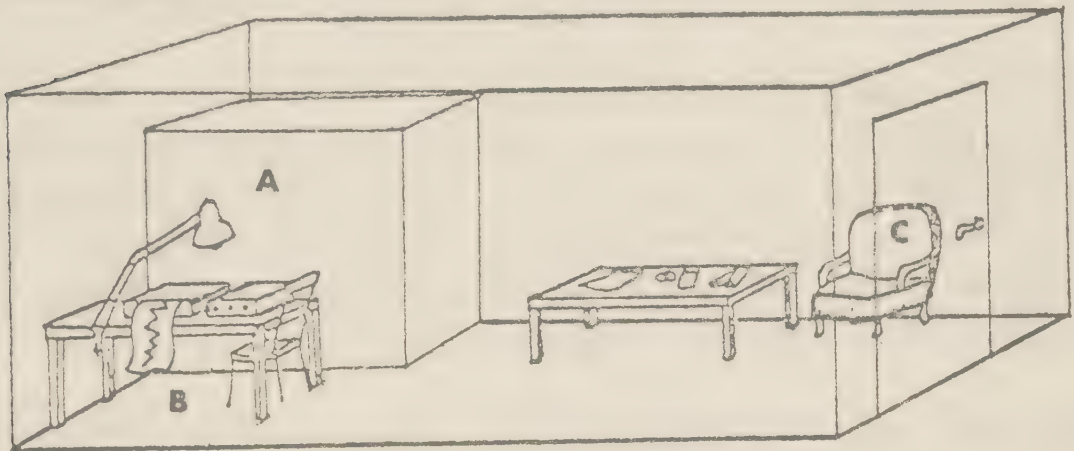
M e t h o d

Design

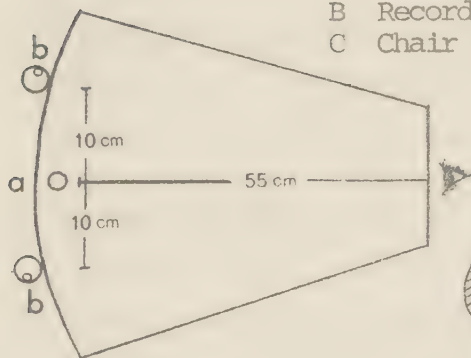
A subject who was adapted to the dark was tested on three different occasions with a test program containing (a) measurement of the level of dark adaptation, (b) cffi with one light-source ($cffi_1$), and (c) cffi with two simultaneous light sources ($cffi_2$). Flicker rate was held constant at 10 cps and the intensities of the lights were systematically varied.

The subjects were randomly divided into three groups and each group was given one tester. The three test occasions were (1) in the morning the first day (8-12 a.m.), (2) in the afternoon the same day (2-7 p.m.), and (3) in the morning the eighth day.

Apparatus



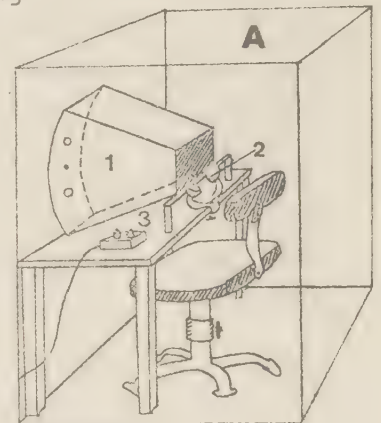
- A Tent where the actual testing takes place.
 B Recorder and control unit.
 C Chair for pre adaptation.



- a Fixation light.
 b Stimulus lights.
 c Chin rest.



- I Ping-pong ball.
 II Diode.
 III 10 mm $\varnothing$ opening.



- 1 Box with stimulus lights.
 2 Chin rest.
 3 Buttons used for indicating responses.

Fig. 1. The set up for the testings and details of the stimulus array and one of the stimulus lights.

An automatic adaptometer (Krakau and Öhman, 1966) has been modified in cooperation with the staff of the department of Ophthalmology at the hospital in Lund. The modified version consists of three parts: a stimulus array, an automatic unit with three built-in programs run in accordance with the responses of the subject and a pen-recorder that continuously records the responses on a paper chart run at a speed of 20 mm/min. The entire set-up is shown in Fig. 1.

As can be seen from this figure the three light sources which make up the stimulus array are placed in the bottom of conical box with the narrow, open end turned towards the subject, who is seated on an adjustable chair with his chin on a chin rest. The distance between the stimulus lights and the subject's eyes is 55 cm.

The light is produced by diodes. A red one is placed level with the eyes of the subject and has a fixed intensity above the threshold for photopic vision and does not excite the rods (Ganong, 1967). It is always turned on and has the function of fixation point during testing. The stimulus diodes are placed one in each of two ping-pong balls and give indirect light through openings 10 mm in diameter. They emit light with the wavelength 5650 Å, perceived in photopic vision as "green blue", but as they are always used at low intensities below the threshold for the cones they are consequently perceived by the subject as "white".

The three built-in test programs are designed to measure the following functions: (i) Level of adaptation to the dark defined as the lowest intensity where the subject can perceive light, (ii) cf_{fi_1} defined as the lowest intensity at which the subject can discriminate between a steady and a flickering light. Only one stimulus light is used here and it alternatively shows flickering and steady light in

a random sequence (iii) $cffi_2$ defined as the lowest intensity at which the subject can discriminate between a flickering and a steady light of equal intensities shown simultaneously but interchanging positions in a random sequence. Flicker rate is always kept constant at 10 cps. In programs (i) and (ii) the stimulus light is situated 10 cm above the red fixation light and in program (iii) the positions of the lights are 10 cm above and under the fixation light respectively.

The subject indicates that light or light of a certain quality (flicker) is perceived by pressing a button. In programs (i) and (ii) only one of two buttons is used and in program (iii) the button that corresponds to the position of the flickering light is to be pressed.

The apparatus has a built-in compound criterion for "correct response", defined as three consecutive correct indications. In program (i) a correct response is to press the button in the presence of light and not to press it when there is no light and in programs (ii) and (iii) it is correct either to press it in the presence of flickering light (program ii) and not in the presence of steady or no light or (program iii) to press the button corresponding to the position of the flickering light and not to press any button when there is no light. When the "correct response" criterion is reached the intensity is automatically decreased by a factor of $\sqrt{2}$. Should an incorrect response occur the intensity is increased by the same step etc.

All three programs start at an intensity level of 0.08 lux. This level is given the numerical value of 1, the next lower level the value of 2 and so on as far as the subject reacts correctly.

The intervals between the termination of one stimulus period and the onset of the next are automatically randomized within the interval 0.5-4.5 sec. A stimulus period lasts 4 sec if no indication is given. If the button is pressed within this period, the light is turned off. Each change of intensity level is recorded and consequently every "correct or false response" is marked.

The stimulus array is placed inside a small tent made of black plastic film. There is no light in the room outside apart from some stray light from the recorder and the program unit (see Fig. 1).

Procedure

During the first five minutes the subject is instructed and the apparatus adjusted to individual requirements. The room is dimly lit by a single light source during this period. Pre-adaptation is then performed during 15 min in complete darkness with the subject seated in a comfortable armchair. The tester is also present in the room. When pre-adaptation is completed the subject is helped over to the tent and placed on the chair with his chin on the rest. He is then instructed to fixate the red light continuously and to press the upper button when a "white" light is seen above the red one. The program is started and run for 2 1/2 minutes and the subject is then instructed to lean back, close his eyes and rest. After 30 seconds he is told to place his chin on the rest again, fixate the red light and to press the button when the "white" light flickers or "quivers" but not to press it when the light is steady. $Cffi_1$ is measured in this way for 2 1/2 minutes followed by another 30 sec pause after which the subject is instructed to press the button that corresponds to the position of the flickering or "quivering" light.

Cffi₂ is measured in this way for three 2 1/2 minute periods with 30 second pauses between them. The third 2 1/2 minute period completes the test. The light is then turned on and the subject is allowed to have a look at the results. Finally a questionnaire is answered regarding drinking and smoking habits and consumption immediately prior to the test. Only the latter question is answered at retest but in other respects the procedure is identical.

Subjects

50 subjects volunteered and were paid 30 SKr for their participation. There were 23 males and 27 females, mostly students, with a mean age of 25.5 years and within the range 12 to 57 years. The subjects were randomly divided into three groups and each group was given a tester (Table 1).

Table 1. Age and sex distribution in the three subgroups.

Group	number males	of females	mean age
I	9	8	25.4
II	7	10	24.8
III	7	9	26.5

Results

The levels of dark adaptation, the cffi for one stimulus light and the cffi two stimulus lights were calculated in two ways: the lowest intensity reached and the mean of the five lowest levels reached (represented by the highest numerical values). All results were calculated as group means and the total sample was divided into subgroups for comparison according to the following scheme: 1. Measurements, 2. Test occasions, 3. The three groups given different testers, 4. Males - females, 5. Glasses - no glasses, 6. Smokers - non-smokers.

Fig. 2 shows the results and 99% intervals of confidence for the total sample calculated over all occasions.

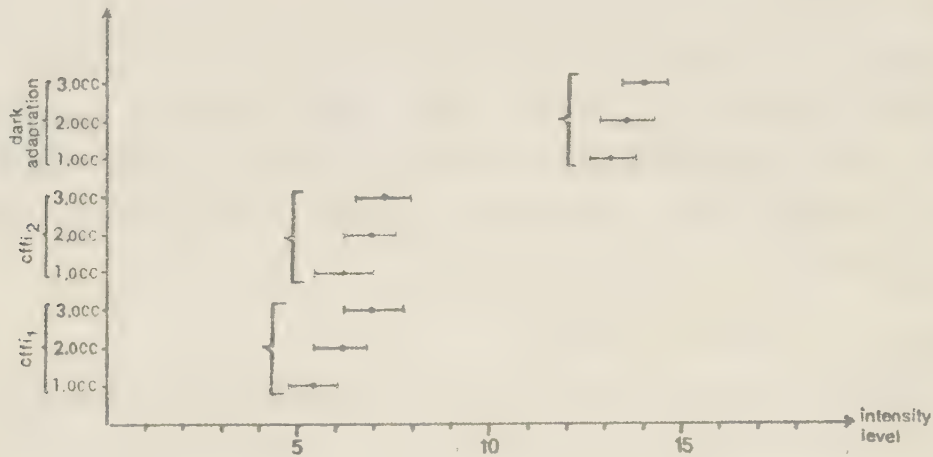


Fig. 2. Results, calculated as the means of the different occasions, and 99% intervals of confidence.

The correlation between the two different ways to determine the lowest level reached is extremely high ($r = 0.99$).

As can be seen in Figs. 2 and 3 there is an improvement, in all measures, over occasions. The difference is significant only for $cffi_2$ on 5%-level ($t_{98} = 2.59$ and $t_{98} = 1.98$).

The correlation between $cffi_1$ and $cffi_2$ was 0.71.

There are differences between the three groups defined by the testers. As can be seen in Fig. 4 these differences include magnitude as well as standard deviation. An analysis of variance showed a significant ($P < 0.02$) tester's effect. However, the absolute magnitude of the differences are very small.

No significant differences could be found between males-females or between glasses-no glasses. ($t_{148} = 0.151$ and $t_{98} = 0.486$ resp.)

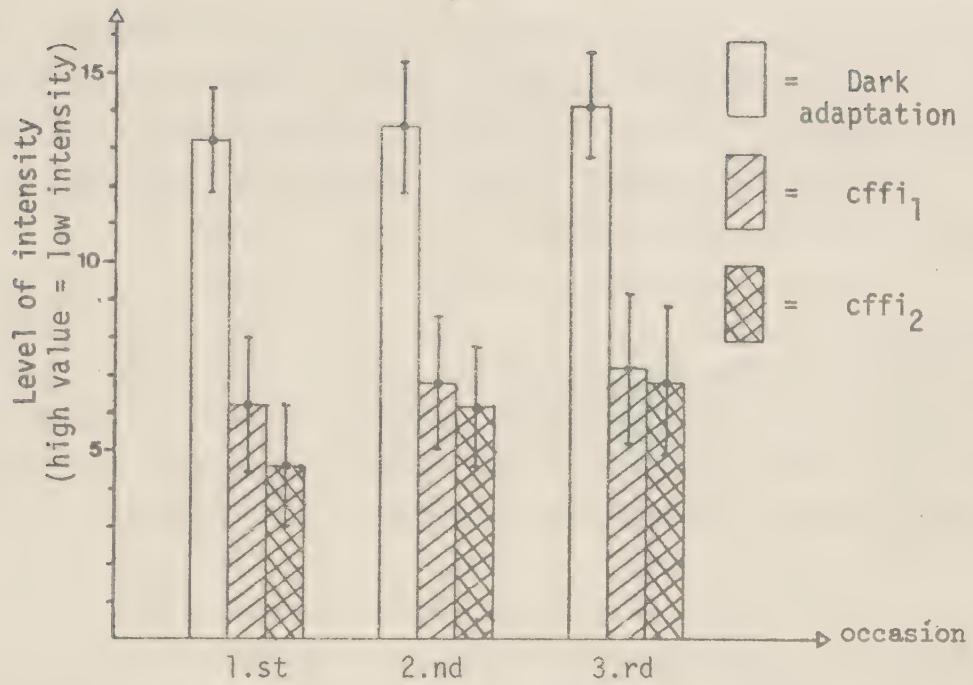


Fig. 3. Results for the total sample on the three different occasions. Standard deviations are marked as thin lines.

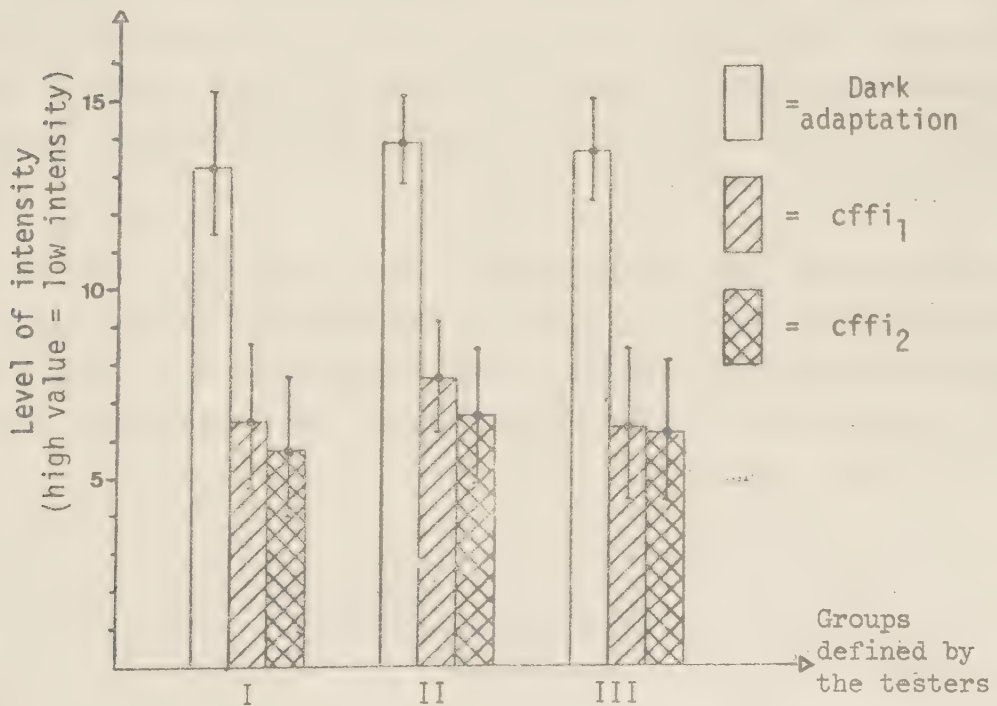


Fig. 4. Results for the three subgroups, defined by the different testers and calculated as the mean of the three different occasions. Standard deviations are marked as thin lines.

In the comparison ($cf\text{fi}_2$) between smokers-non-smokers no significant differences could be found within any of the occasions. However, in every case the differences, although small, were in the favour of the non-smokers, saying that they were able to discriminate at lower intensities.

Discussion

The purpose of this investigation was to obtain data upon which a standardized procedure could be based. Further the stability of the test was to be measured. Lastly the results were to be treated as normative if possible.

One criterion put forward in connection with a standardized procedure was that of simplicity in handling the apparatus and instructing the subject. One way to reach this criterion is to reduce the number of measurements in the test. The most tedious and the same time the most difficult phase, both when instruction and performance are considered, is the measurement of $cf\text{fi}_2$. If this could be excluded from the test, the above-mentioned criterion could be more easily reached.

The correlation between $cf\text{fi}_1$ and $cf\text{fi}_2$ was high and in connection with the fact that the absolute magnitude of the difference was relatively small and that $cf\text{fi}_1$ and $cf\text{fi}_2$ are based on the same principle, it is safe to exclude the measurement of $cf\text{fi}_2$ from the test.

When the results from the pen recorder are transformed to numerical values, the more speedy procedure of marking the lowest level of intensity reached is quite sufficient. This assumption is based on the very high correlation between this method and the method of calculating the means for the five lowest levels reached.

There were no significant differences between males-females, glasses-no glasses and smokers-non smokers within occasions. Only for the smokers-non smokers could a significant difference (on the 5%-level) be found between occasions.

An over-all comparison between occasions revealed a slight improvement over time. This trend was significant only for $cffi_2$ and on a 5%-level, and even here the absolute magnitude is small. When the three groups defined by the testers were compared a difference was found. An analysis showed that this was to be considered as a tester effect. Here as well the absolute magnitude of the differences was small.

The generally small size of found differences in connection with the satisfactory narrowness of the 99% intervals of confidence confirms the stability of the method, all programs included.

It is to be observed that all results were obtained in a situation where the stimulus is not correlated to the optimal sensitivity of the rods. The diodes used emit the wavelength 5650 Å (perceived as "green-blue" at intensities above the threshold for the cones) whereas the rods are most sensitive to the wavelength 5000 Å (pure blue). Technical reasons guided the choice of the diodes used - there are no diodes with shorter wavelengths commercially available.

When it comes to the question of whether these results can be considered normative, different facts point in that direction: the relatively small deviations in spite of the fact that the sample is heterogeneous and the fact that the trend towards improvement over time was an over-all trend

However, the sample is relatively small, and further investigations of samples from a non-exposed population should be

performed and the results compared to the present ones. All normal variations are of magnitudes smaller than the predicted ones caused by MeHg induced lesions (Berlin, Gage, Grant, Hellberg and Skerfving, 1976). This prediction is partly confirmed by results obtained in an earlier investigation on monkeys (Hellberg and Hellström, 1977).

A standardized test procedure should consist of Instruction, Pre-adaptation, Measurement of level of dark adaptation and measurement of cffi. The reliability is sufficient. To investigate the normative value of the present results, further tests should be performed on samples from a non-exposed population. The question of what could be gained by changing the wavelength of the stimulus light toward the optimum of the rods should be investigated.

Effects of fatigue by a full working day should be investigated since in an epidemiological investigation with this method used as a diagnostic instrument, circumstances cannot be expected to be as favorable as in the present investigation.

A c k n o w l e d g e m e n t s

This work was supported by a grant from the Swedish Medical Research Council (Project No 2081).

The analysis of variance was performed by Bo Gullberg, MA, the Department of Statistics, University of Lund.

R e f e r e n c e s

- Berlin, M., Grant, C.A., Hellberg, J., Hellström, J. & Schütz, A. (1975). Neurotoxicity of Methylmercury in Squirrel Monkeys. Arch. Environ. Health, 30, 340-348.
- Berlin, M., Gage, J.C., Grant, C.A., Hellberg, J. & Skerfving, S. (1976). Fortsatta undersökningar över metylkvicksilvers neurotoxicitet och biotransformation samt utarbetandet av metoder för studier av toxiska effekter. Forskningsplan och Vetenskaplig Rapport, B75-14X-2081-09, Dpt. of Environ. Health, Lund University.
- Chandler, P.J., Parsons, O.A. & Haase, G.R. (1964). Identification of Brain Damage by Flicker Perimetry: a Second Cross-validation. Perceptual and Motor Skills, 19, 217-218.
- Ganong, W.F. (1967). Rewiev of Medical Psychology. Los Altos: Lange Medical Publications. Pp. 96.
- Hellberg, J. & Hellström, J. (1977). Changes in Scotopic Flicker Fusion Intensity (cffi) in Squirrel Monkeys Exposed to Methyl Mercury. Psychol Res. Bull., Lund University, 17, No. 12.
- Krakau, T. & Öhman, R. (1966). An Automatic Adaptometer. Försvarsmedicin, 4, 184-189.
- Parsons, O.A. & Huse, M.M. (1958). Impairment of Flicker-discrimination in Brain-Damaged Patients. Neurol., 8, 750-755.
- Parsons, O.A. & Gottlieb, A.L. (1960). Visual Field Impairment in Brain Damage: Cross-Validation and Reliability of a Method of Flicker Perimetry. AMA Arch. Ophtal., 63, 1009-1014.
- Rosenblum, L.A. & Cooper, R.W. (Eds.) (1968). The Squirrel Monkey. New York: Academic Press.

PSYCHOLOGICAL RESEARCH BULLETIN

XVIII:2 1978

*The Influence of Fatigue and Different Light
Wavelengths on Critical Flicker Fusion
Intensity in Scotopic Vision*

by Jan Hellberg



LUND UNIVERSITY SWEDEN

A b s t r a c t . - Measured cffi in scotopic vision and the level of dark adaptation by using a modified, automatic adaptometer. Flicker was held constant at 10 cps. Light of two different wavelengths (5650 Å and 5900 Å) was used. The subjects (22 males and 27 females) were mainly university students that volunteered for the test. Cffi for the two wavelengths was compared to an earlier investigation where 5650 Å had been used. The results showed that time of the day did not influence cffi. A significant increase of cffi occurred when the wavelength was changed from 5650 Å to 5900 Å. An extrapolation was made that pointed to a 72%-gain in sensitivity of the apparatus if one could use a light source which emits the wavelength 5000 Å. This was considered important since the apparatus is meant to be used as a diagnostic instrument in epidemiological investigations of populations exposed to methyl mercury (MeHg).

It has been shown that squirrel monkeys (*Saimiri sciureus*) orally exposed to methyl mercury (MeHg) show an increase in critical flicker fusion intensity (cffi) in scotopic vision before any conventional clinical signs have been observed (Hellberg and Hellström, 1977). The change in cffi is caused by lesions in the visual cortex (Berlin et al., 1975).

These results were of such significance that it was felt that the method could be employed as a diagnostic instrument for use on human subjects as well. An automatic adaptometer (Kraakau and Öhman, 1966) was modified to allow measurements of cffi in scotopic vision at the wavelength 5650 Å. Tests were performed on human subjects in order to obtain normal values and their variations and to standardize the test procedure (Hellberg, 1978).

However, two questions were not answered satisfactorily in the previous investigation: (1) To what extent does the fatigue produced by a full working day influence the cffi, and (2) how would the sensitivity of the method be affected by using other wavelengths of light?

The last question is connected to the way the test apparatus is constructed. Diodes are used as light sources, functioning as fixation- and stimulus lights. Due to technical limitations, diodes with shorter wavelengths than 5650 Å are not commercially available. At intensities above the threshold for photopic vision (daylight vision) this wavelength is perceived as green-blue. Below this intensity only the rods are active (scotopic vision) and the light is perceived as "white". In this investigation only intensities below the threshold were used, and consequently the stimulus was perceived as "white" in all tests.

The optimum for rod sensitivity corresponds to the wavelength 5000 Å as can be seen in the diagram of spectral sensitivity for scotopic vision (Fig. 1). At intensities above the threshold for photopic vision this wavelength is perceived as pure blue. As a consequence of using diodes as stimuli the test is limited to a region well below the optimum. However, this limitation was initially accepted since the use of diodes is of such an advantage in that it permits an exact control of both intensity and flicker rate without any extra equipment.

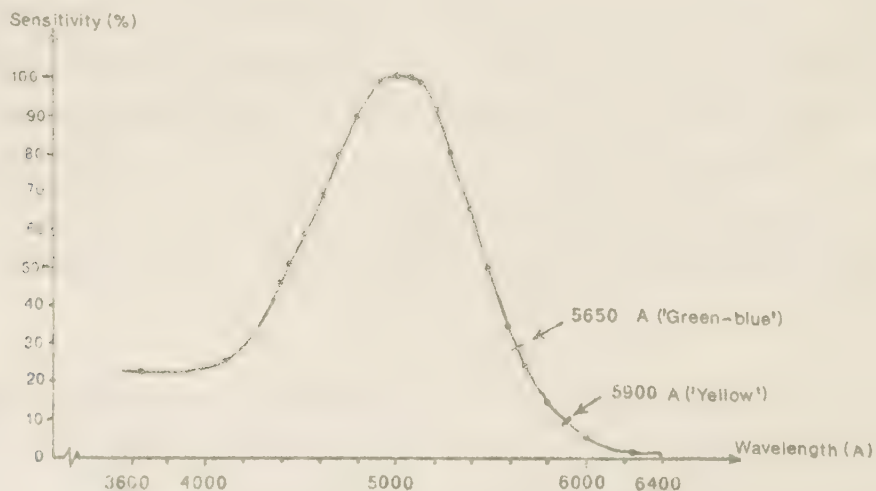


Fig. 1. Spectral sensitivity of human rod vision (scotopic vision). The wavelengths of the two stimulus lights discussed are specially marked (Adapted from Morgan, 1965).

The present investigation involve two available wavelengths (5650 A and 5900 A) from which prediction about the optimum (5000 A) sensitivity is made. In addition a follow-up of the study previously mentioned (Hellberg, 1978) is made concerning the specific question of how much fatigue influences cffi. Both questions are essential since the apparatus is meant to function as a diagnostic instrument in epidemiological investigations of populations exposed to MeHg. If these exposures occur in countries like Iraq (where about 30,000 individuals were exposed orally in 1972) the tests must be performed outside the laboratory and on individuals subject to fatigue and vitamin A malnutrition. The latter is known to affect scotopic vision negatively (Ganong, 1967). A diagnostic instrument must be sensitive enough to measure cffi for scotopic vision despite rather severe vitamin A deficiencies.

M e t h o d

The subject who was fully dark adapted was tested for level of dark adaptation and cffi for two different light wavelengths: 5650 A (cffi₅₆₅₀) and 5900 A (cffi₅₉₀₀) respectively. Level of dark adaptation was defined as the lowest intensity at which the subject perceived light with the wavelength 5650 A and cffi as the lowest intensity at which the subject could discriminate between a steady and a flickering light (10 cps) with the wavelengths 5650 A and 5900 A alternately. The first measure was obtained in order to control that the subject possessed normal scotopic vision.

Apparatus

The apparatus was automatic and could be set to run either of two programmes. The first measured level of dark adaptation and the second cffi at a fixed flicker rate of 10 cps. A red light diode with an intensity above the threshold for photopic vision was constantly lighted and functioned as fixation point. The red light did not affect dark adaptation. Two light

diodes were placed opposite each other inside a ping-pong ball, which was painted black, and were giving indirect stimulus light through a 10 mm $\varnothing$ opening. The diodes emitted 5650 Å and 5900 Å respectively, and could be interchanged any time during the test but could not be turned on simultaneously.

The three light sources were mounted in the bottom of a conical box with the narrow, open end turned toward the subject. The distance from the eyes of the subject to the stimulus and the fixation lights was 55 cm, and the stimulus light was situated 10 cm above the fixation light. There was also a chin rest for convenient fixation of the head of the subject. By pressing a button the subject indicated that light or flicker was perceived.

The built-in criterion for "correct response" was three consecutive correct markings which meant that the subject pressed the button when light or flicker actually was present and did not press it when, in the first programme, no light was present or, in the second programme, when a steady light or no light was present. A "correct response" meant that the light intensity was automatically decreased with the factor $\sqrt{2}$. This continued until a "wrong response" occurred. When this happened the intensity was increased by the factor $\sqrt{2}$. Every change of intensity level was recorded on a pen-recorder and each step was given a numerical value from 1 and upwards, where 1 stands for the initial level (.8 lux).

The intervals between the onsets of the stimulus light were randomized as was the sequence steady-flickering light. If the button was not pressed the stimulus light was turned on for 4 seconds. If the button was pressed within this period, the light was turned off immediately. (For a more detailed description of the apparatus, see Hellberg, 1978.

Procedure

All tests were performed on working days in the evenings between 5-9 pm. When entering the room the subject was instructed to adjust the chair and the chin rest to a comfortable position and at the same time was told the working principles of the apparatus. This lasted for about five minutes and during this time the room was dimly lit. A pre-adaptation for 15 minutes followed in complete darkness with the subject seated in an armchair. The experimenter remained in the room.

When the pre-adaptation was completed the subject took his place in front of the apparatus, was told to place his chin on the chin rest, to fixate the red light and to press the button when a white light was seen above the fixation light.

The apparatus was turned on and the first programme run for $2\frac{1}{2}$ minutes after which the subject was told to lean back, close his eyes and relax. During a 30 seconds pause the apparatus was set to run the second programme and after the pause the subject was given new instructions saying that the button should be pressed only when the white light flickered or "quivered". The apparatus was turned on and the programme run for three $2\frac{1}{2}$ minute periods with 30 second pauses in between.

In the second programme cffi_{5650} and cffi_{5900} were measured alternately according to a random sequence made up for the entire sample. This meant that a subject could be tested with one of the possible combinations of two lights and three measurements. The sequence resulted in 66 measurements of cffi_{5650} and 88 of cffi_{5900} . Total time for testing one subject was about 30 minutes including the time needed to fill in a questionnaire stating the number of active hours prior to the test as well as the amount of tobacco and drugs consumed

Subjects

22 males and 27 females, mostly students at the university, volunteered and were paid 15 Swedish crowns for their participation. The mean age was 22.8 ranging from 11 to 46 ($s=4.37$). The mean number of active hours before the test was 9.5 ($s=1.86$).

Results

Mean value and standard deviation for level of dark adaptation were calculated and compared to the corresponding values from an earlier investigation ($m=13.7, s=1.83$ and $m=13.6, s=1.55$ respectively). No significant differences could be found ($t_{197} = .36$).

Mean value and standard deviation for cff_{5650} were calculated and compared to the corresponding values from the earlier investigation with the same stimulus light and obtained during testings between 8-12 am ($m=5.83, s=2.11$ and $m=6.16, s=1.91$ respectively). No significant differences could be found ($t_{214} = 1.11$).

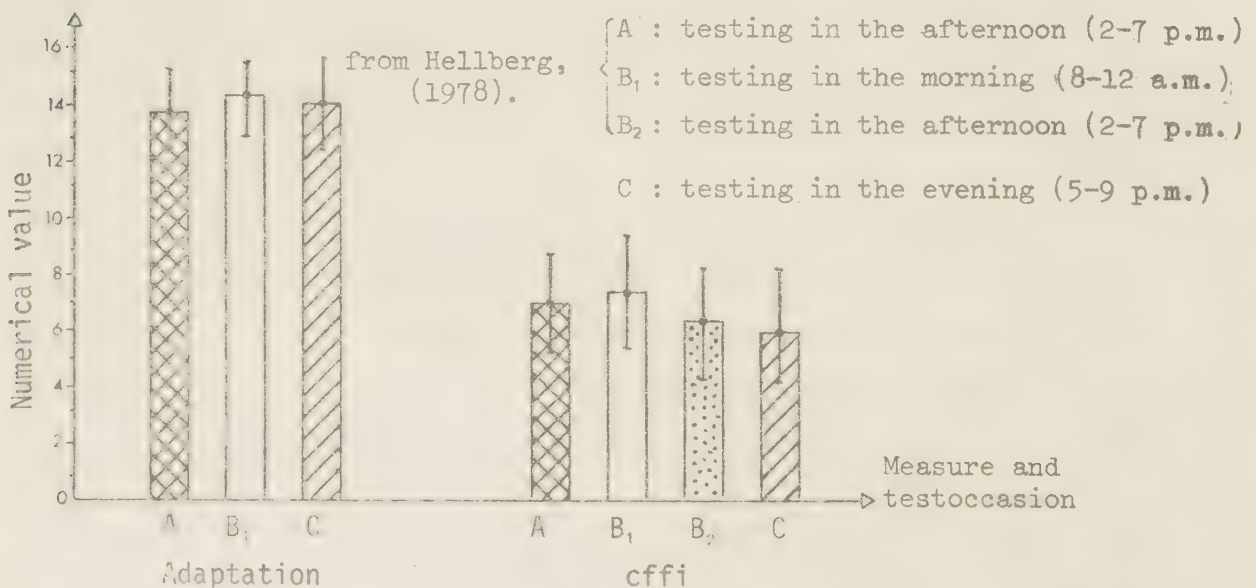


Fig. 2. Comparison of results of the present investigation (C) and earlier one (A, B₁ and B₂) in order to discover possible effects of fatigue.

Corresponding values for cffi_{5900} were $m = 2.47$ and $s = .92$.

Comparison between cffi_{5650} and cffi_{5900} showed a significant difference on the .001-level ($t_{35} = 10.99$).

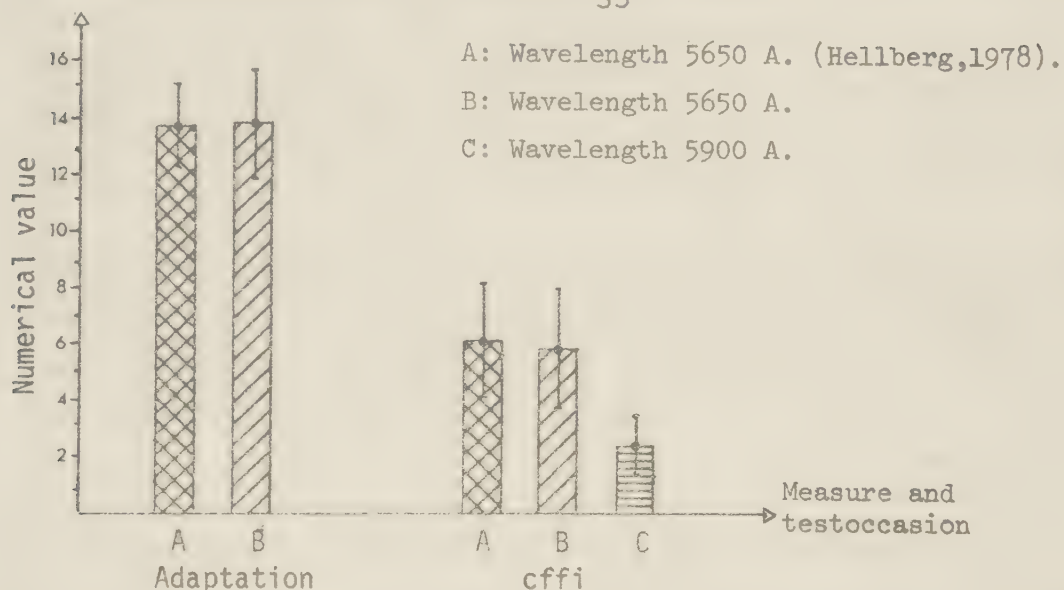


Fig. 3. Sensitivity to two different wavelengths obtained in the present and an earlier study.

Figures 2 and 3 illustrate the results from the present and the earlier investigations. They differ very little in magnitude when the comparison is made for 5650 A. As shown by the absorption spectrum in Fig. 1, the loss of sensitivity was 19% when changing from 5650 A to 5900. The mean difference $\text{cffi}_{5650} - \text{cffi}_{5900}$, as measured by our apparatus, is 3.57 steps (each step is a change in intensity by the factor $\sqrt{2}$). The magnitude of this difference should be compared with the above mentioned loss of 19%. The absorption spectrum showed a 72% loss of sensitivity when 5650 A was compared with 5000 A. Thus, an extrapolation based on this would predict a gain, expressed numerically, from 5.8 to 12.7 for cffi_{5000} .

D i s c u s s i o n

The comparison between the group tested in the present inves-

tigation and the one tested in the earlier one, shows that fatigue, within normal boundaries, does not influence either the level of dark adaptation or cffi. The present sample as well as the earlier one is considered representative for the normal population within the range 11 - 50 years of age. A practical consequence of this is that the use of the apparatus as a diagnostic instrument is not restricted to a certain time of day.

The fact that there are no differences between the present and the earlier results means that they can be considered normative and consequently can be used as references when results obtained in a clinical situation are analyzed for deviations.

A highly significant loss of sensitivity is associated with a relatively small change of wavelength (5650 Å to 5900 Å). Even if a strict extrapolation is not possible since there are only two sets of data related to two different wavelengths, the predicted gain of 72% clearly indicates, that it would be possible to make the test much more sensitive by changing the stimulus light towards optimum for rod sensitivity.

However, the modifications necessary for such a change would mean a major change of the apparatus. This is due to the earlier mentioned fact that there are no diodes with shorter wavelengths than 5650 Å commercially available. From a technical point of view the change of wavelength must therefore be based on a white light with fixed intensity, a monochromatic filter and a motor driven aperture or a graded gray-filter. The flicker must be produced by a rotating disc with matched speed and number of holes. It is of course technically possible to construct an apparatus in accordance with

these principles, but the solution would be both clumsy and expensive. When the technical difficulties are not considered, it is clear that the convenient test procedure, with a minimum of instructions required, would not be affected.

Generally speaking it is preferable, when looking for the perception of small changes, to use stimulus intensities as near a lower threshold as possible. This is based on the well known fact that the changes in stimulus intensities required for detection are smaller the lower the absolute magnitude of the judged stimulus. To find out if this general principle holds in this case, it is necessary to make another investigation with a modified apparatus.

As mentioned earlier it is known that vitamin A deficiencies affect scotopic vision negatively. In view of this and knowing that epidemiological investigations with the method might have to be performed on populations suffering from malnutrition, it is also in this respect essential to construct an instrument as sensitive as possible. However, this does not necessarily mean reaching the maximum. Optimum for the method might be considerably below this level, but with the present and earlier results from a normal population available this is only speculations. Further testings of populations comparable with those described above have to be performed in order to establish an optimal level of sensitivity of the apparatus.

A c k n o w l e d g e m e n t

This work was supported by a grant from the Swedish Medical Research Council (Project No 2081).

References

- Berlin, M., Grant, C.A., Hellberg, J., Hellström, J., & Schütz, A. (1975). Neurotoxicity of methyl mercury in Squirrel monkeys. Arch. Environ. Health., 30, 340-348.
- Ganong, W.F. (1967). Review of medical psychology. Los Altos: Medical Publications.
- Hellberg, J. & Hellström, J. (1977). Changes in scotopic flicker fusion intensity (cffi) in Squirrel monkeys exposed to methyl mercury. Psychol. Res. Bull., Lund Univ., 17, No. 12.
- Hellberg, J. (1978). A method for measurement of critical flicker fusion intensity (cffi) in scotopic vision. Psychol. Res. Bull., Lund Univ., 18, No. 1.
- Krakau, T., & Öhman, R. (1966). An automatic adaptometer. Försvarsmedicin, 4, 184-189.
- Morgan, C.T. (1965). Physiological psychology. New York: McGraw-Hill.

